Journal of Plant Growth Regulation
https://doi.org/10.1007/s00344-024-11401-z

Involvement of Nitric Oxide in Biochemical and Physiological Response of Potato Seedling Under Cold Stress

Jafar Nabati[1] · Zahra Nemati[1] · Elaheh Boroumand Rezazadeh[1]

Received: 12 March 2024 / Accepted: 15 June 2024

Abstract

Cold temperatures harm tropical crops, but understanding how molecular signals help plants cope could aid in climate change adaptation. This study tested if sodium nitroprusside (SNP), a nitric oxide (NO) donor, could improve potato tolerance to cold. Potato seedlings, treated or non-treated (0.5 mM) with SNP, were exposed to cold stress (0 and −2 °C) for 6 h. The study was conducted in a completely randomized design, incorporating three factors in three replications. Results showed that cold stress reduced physiological and biochemical parameters in all seedlings, but less so in those treated with SNP. SNP treatment boosted physio-biochemical activity and increased levels of soluble sugars and enzymatic and non-enzymatic antioxidants. Seedlings treated with SNP and exposed to cold stress had lower levels of H_2O_2 and malondialdehyde, suggesting that NO may alleviate the harmful effects of cold. The analysis conducted using PCA demonstrated correlations between variables and treatment groups. Notably, the first two principal components (PC1 and PC2) accounted for 77.6% and 78.1% of total variance, respectively, under both 0 and − 2 °C temperatures. Under temperatures below 0 °C, the results of the factor analysis (FA) revealed that PC1 exhibited the highest distribution of data, containing the most prominent variation in Squared cosine values (SCV) values at 0.79. Among the variables, Electrolyte leakage (EL) had the best representation in PC1, with the corresponding maximum SCV values at 0.78 under − 2 °C. This result highlights the potential use of SNP in manipulating cold tolerance in potato plants.

Keywords Low temperature · Principal component analysis (PCA) · Sodium nitroprusside (SNP) · Squared cosine values (SCV)

Introduction

Adverse environmental conditions have a negative impact on crop plant growth, development, productivity, and geographical distribution. Low temperatures (LT) represent some of the most severe abiotic stressors that can jeopardize the yields of crops (Sanchez-Vicente et al. 2019; Zhang et al. 2021). Low temperatures can create a disparity between generation and elimination of reactive oxygen species (ROS). Overproduction of ROS may cause oxidative damage through peroxidation of membrane lipids, resulting in leakage, and loss of chlorophyll (Ding et al. 2020). In this context, the scavenging of these deleterious ROS is vital for plant survival, which is achieved principally by antioxidants. Plants possess a responsive antioxidant protection system consisting of both enzymatic and non-enzymatic antioxidants that aid in absorbing and neutralizing the excess ROS (Mao et al. 2018). Furthermore, there is evidence to suggest antioxidant systems also shield plants from oxidative damage induced caused by LT stress (Esim and Atici 2014). Alternatively, under extreme stress, the adverse effects of oxidative damage may not be adequately reduced by the antioxidant system (Hussain et al. 2018).

Plants necessitate changes to their photosynthesis capacity to withstand under stress conditions. One possible approach is regulate several gas exchange features, including stomatal conductance (Gs), transpiration rate (E), and intercellular CO_2 concentration (Ci) (Shi et al. 2021).

Improvement in plant defense responsiveness can mitigate damage caused by LT. Such enhancement is attainable with the application of exogenous signalling molecules

Handling Editor: Nicola Busatto

✉ Jafar Nabati
jafarnabati@um.ac.ir

[1] Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

Published online: 02 July 2024

(Anelia 2017), which are produced upon the detection of environment stress. Subsequently, these molecules amplify the stress signal and initiate downstream responses (Longo et al. 2018). Augmenting the plant's tolerance to harsh conditions is possible by absorbing exogenous small signalling molecules as it can increase endogenous levels, stimulate relevant metabolic processes, and elevate the plant cold tolerance (Zheng et al. 2021).

Nitric oxide (NO) is a bioactive compound that regulates numerous plant physiological processes, including seed germination, gene regulation, augmentation of antioxidant enzyme activity, and response to abiotic stress (Fancy et al. 2017). Furthermore, NO has a suitable structure to neutralize toxic ROS in plant cells via electron exchange (Fancy et al. 2017; Nabi et al. 2019). NO also participates in controlling various responses to cold stress and alleviating low-temperature stress for plants (Liu et al. 2019). Additional research findings show the application of exogenous NO promotes the prevention of cold damage in many agricultural crops (Esim and Atici 2014; Fan et al. 2015; Wu et al. 2022; Diao et al. 2022; Sohag et al. 2020).

Cultivated potato (*Solanum tuberosum* L.), the fourth most important food crop in the world after *Oryza sativa* L., *Triticum* spp., and *Zea mays* L., is grown worldwide for its high nutritional value (Faostat 2022). Freezing stress poses a noteworthy obstacle to potato production in many areas of the world, as shown by multiple previous studies (Palta et al. 1997; Li et al. 2015). Most potato cultivars cease growing at less than 7 °C, experience injury from cold below −0.8℃, bear frost at less than −1.5℃, and undergo freezing at less than −3℃ (Palata and Li. 1979; Chen and Li 1980). The impact of low-temperature conditions on potato seedlings including the expression of genes and microRNAs, plasma membrane lipid, and antioxidant activity was investigated (Palta et al. 1993; Mora-Herrera, M.E. and Lopez-Delgado 2007; Rensink et al. 2005; Yan et al. 2021).

Numerous studies have investigated the role of NO in regulating physiological responses of plants under stress, spanning a range of species (Esim and Atici 2014; Fan et al. 2015; Wu et al. 2022; Diao et al. 2022; Sohag et al. 2020). However, there has been no investigation into the effects of NO on potato plants subjected to cold stress, including chilling and freezing. The present study aims to examine the effects of chilling and freezing stress on physiological and biochemical aspects and processes in potatoes under SNP treatment. These include levels of photosynthetic pigments, both enzymatic and non-enzymatic antioxidants, and secondary metabolites.

Since, NO is a compound that reduces cold stress damage in plants. The objectives of the research were to assess the detrimental effects of LT on the physiological and biological characteristics of potato cultivars under chilling and freezing stress, and to improve their cold tolerance through the application of NO.

Materials and Methods

Plant Materials and Treatments

Twelve tetraploid cultivars of potato cultivars ((1) Adato, (2) Agria, (3) Croatia, (4) Donata, (5) Fontane, (6) Karelia, (7) Paradiso, (8) Ricarda, (9) Royal Blue, (10) Sante, (11) SanteT (While visiting the farm, it was noticed that out of 11 the different cultivars, only a single plant of the Sante managed to survive the cold weather during autumn, a sample was taken from this plant and then propagated using tissue culture), and (12) Vogue) were selected for this study. Potato seedling 35 day, derived from tissue culture, were placed into 10 cm diameter plastic pots filled with a mixture of perlite and cocopeat in the ratio a 1:1. Sodium nitroprusside (SNP) served as a NO donor. After five weeks, the seedlings were sprayed with 50 µM SNP for three consecutive mornings. The seedlings were subsequently categorized into treatments listed below: (1) Control 1 (C) was sprayed H_2O and maintained at a temperature of 24 °C. (2) Control 2 (C-SNP) was sprayed with 50 µM SNP and maintained at a temperature of 24 °C. (3) The plants were sprayed with H_2O and subjected to cold stress at temperatures of 0 °C and −2 °C. (4) The plants were spray with 50 µM SNP and subjected to cold stress at temperatures of 0 °C and −2 °C. The cold treatment was performed in a thermogradient freezer, with the environmental conditions gradually adjusted at a rate of 2 °C per hour. The relative humidity inside the freezer was 80–90% with complete darkness. Once the desired temperatures of 0 and −2 °C were reached and maintained for 6 h, the plants were placed in the greenhouse. All physiological and biochemical parameter discussed in this investigation were assessed on the third leaf prior to and following exposure to cold treatment.

Electrolyte Leakage (EL)

For the electrolyte leakage (EL) test, the amount of leaf electrolyte leakage after cold exposure using the Jenwey model 4510 electrical conductivity meter from UK was measured. The EL value was calculated using the following equation proposed by Teutonica et al. (1993)

$$EL(\%) = \frac{EC1}{EC2} \times 100$$

where EC_1 is the initial conductivity, and EC_2 is the final conductivity (boiled – Killed samples).

Relative Water Content (RWC)

Relative water content as reference for assessing plant water status of plants was estimated according to Sharp et al. (1990). RWC values were calculated through the following equation:

$$RWC\% = \frac{(freshweight - dryweight)}{(Turgidweight - dryweight)} \times 100$$

Photosynthetic Pigment, Carotenoids, Flavonoids Content and Anthocyanin

Extraction of chlorophylls (Chl) was implemented from a proportional amount of fresh potato leaves in 96% ehanol. Chla, Chlb, and carotenoids (Car) were quantified using the method described by Dere et al. (1998). Flavonoid content (FC) was measured using the method developed by Chang et al. (2002). Anthocyanin concentration was assessed according to Wagner's (1979) method.

Proline content (PC), total phenolic compound (TPC), total soluble sugars (TSS), and DPPH (2,2–diphenyl–1–picrylhydrazyl)

The estimation of PC was based on previous reports by Bates et al. (1973). TPC was determined using the Folin-Ciocalten reagent as described by Singleton and Rossi (1965). Leaf TSS was determined using Dubois et al. (1951) procedure. The DPPH content method of potato leaves was measured using Abe et al. (1998) method with minor modifications.

Malondialdehyde (MDA) and H_2O_2

Lipid peroxidation of the leaves was quantified by calculating the amount of MDA produced through the thiobarbituric acid (TBA) reaction as per the methodology outlined by Stewart and Bewley (1980). The H_2O_2 content in the potato leaves was extracted and measured using the Sergiev et al. (1997) method.

Antioxidant Enzyme Activities

To determined antioxidant enzyme activities, 0.1 g of fresh leaves from potato seedling were homogenized in solution of 0.1 M potassium phosphate buffer (pH 7.8) containing 0.1 M EDTA. The homogenates were centrifuged at 12,000 ×*g* for 20 min at 4 °C. The resulting supernatant was utilized to analyze the activity of CAT (Velikova et al. 2000), POD (Srinivas et al. 1999), and APX (Yamaguchi et al. 1995) via a spectrophotometer (Jenway UV–Visible Spectrophotometer Model 6305, UK).

Measurement of Leaf Net Photosynthetic Rate

Measurements of the net photosynthetic rate (Pn), transpiration rate (E), stomatal conductance (Gs), and intercellular CO_2 concentration (Ci) conducted by portable gas analyzer device (ADC, Hoddeston UK, model LCA4) under saturating light (1000 $\mu mol^{-2}\ s^{-1}$).

The Yield and Its Components

Number and weight of potato tubers per plant were recorded 30 days after cold treatments.

Statistical Analysis

ANOVA analysis was conducted using Minitab 16.0. In addition, normalized values were used to generate a PCA, correlation, and heat map Squared cosine values (SCV) are used to visually represent the strength of variables contribution to the principal component analysis (PCA) axes. In this study, SCV and PCA were opted for analysis. This approach minimizes potential misinterpretations during result analysis. By utilizing squared cosine and 1-cosine, the resulting linked variance is distinguished from unconnected variance. It is noteworthy that squared cosines, also referred to as *R*-squared (R^2) or the correlation coefficient, commonly fall between 0 and 1. These values provide valuable information about the proportion of variation in the data that can be attributed to independent variables. Thus, they are essential in achieving optimal data fitness. Effectively utilizing SCV in PCA visually highlights the impact of particular variables, reducing the possibility of misinterpretations and aiding comprehension of independent variable importance in explaining total variance in the dataset. The R environment (4.2.2) was utilized to conduct this analysis. Stepwise regression tests were performed to establish the degree of correlation and identify the degree of relation between the weights of cold-stressed potato tubers per plant.

Results

Assessment of Treatment–Variable Interaction via Hierarchical Clustering and Heat Map Analysis Under Normal and Stress Conditions

The physiological and biochemical parameters collected under normal and cold stress conditions (0 °C and −2 °C) were averaged for each individual treatment (C, CS, C−SNP

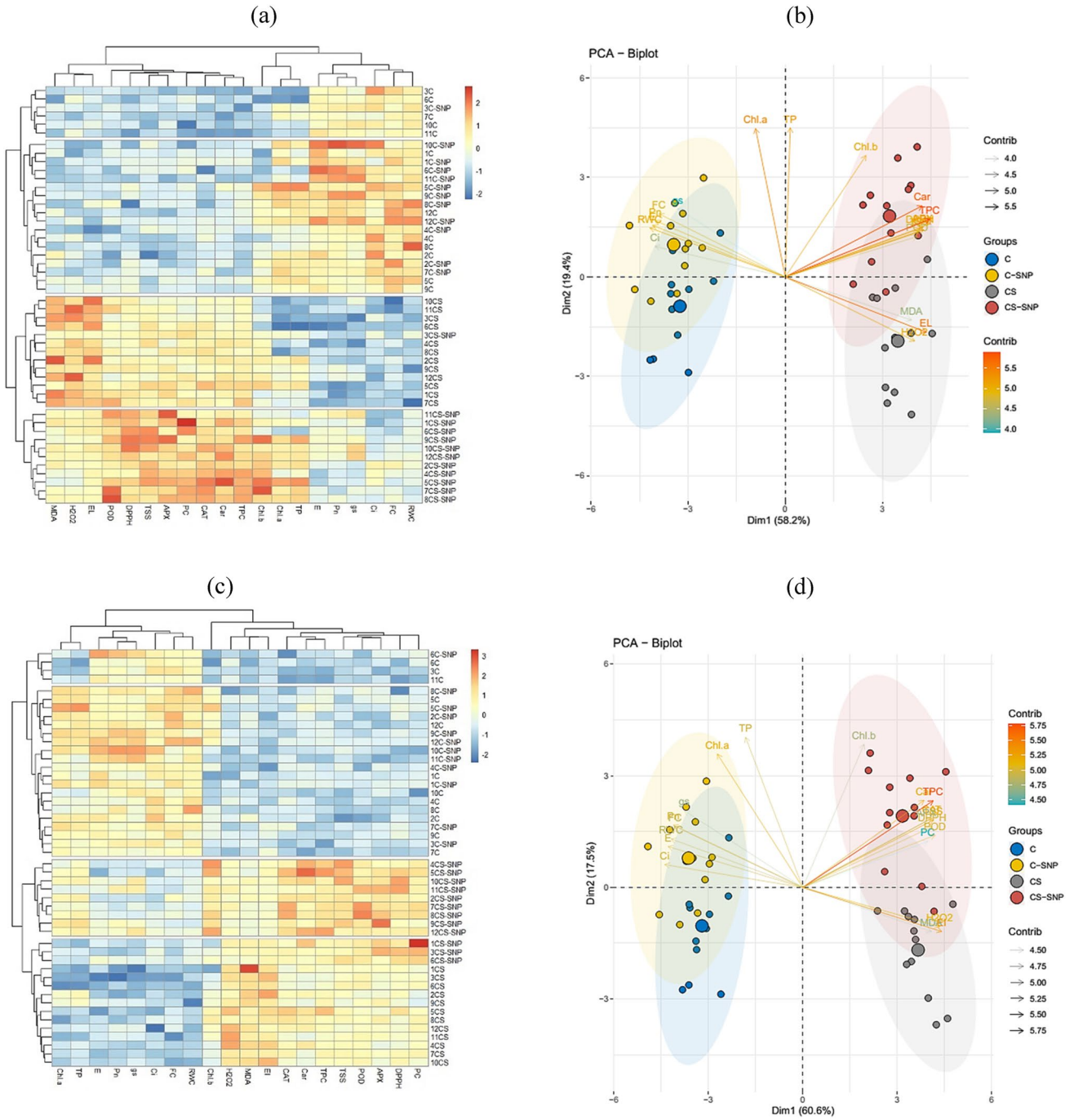

Fig. 1 **a** Hierarchical clustering with heat map and **b** principal component analysis (PCA) under 0 °C and **c** hierarchical clustering with heat map and **d** principal component analysis (PCA) show treatment–variable relationships under − 2 °C. *EL* electrolyte leakage, *RWC* relative water content, *Chla* chlorophyll a, *Chlb* chlorophyll b, *TP* total chlorophyll, *Car* carotenoids, *FC* flavonoid content, *PC* proline content, *TPC* total phenolic compound, *TSS* total soluble sugars, *DPPH* 2,2-diphenyl-1-picrylhydrazyl, *MDA* malondialdehyde, *CAT* catalase, *POD* peroxidase, *APX* ascorbate peroxidase, *Pn* net photosynthetic rate, *E* transpiration rate, *Gs* stomatal conductance, *Ci* intercellular CO_2 concentration, and H_2O_2 hydrogen peroxide

and CS−SNP), and underwent to hierarchical clustering, heat map analysis, and PCA (Fig. 1).

Evaluation of Treatment-Variable Interaction Under 0 °C Based on Hierarchical Clustering and Heat Map Analysis

Hierarchical clustering revealed four clusters related to variable levels at 0 °C (Fig. 1a). RWC, FC and various photosynthetic parameters (Ci, Gs, Pn, and E) were clustered together in first cluster. The heat map demonstrated a reduction in the levels of RWC, FC, CI, Gs, Pn and E under cold stress (CS), but revealed an increase in the levels of cold-stressed plants treated with NO (CS-SNP). In the second cluster, the variables Chla, Chlb and TP were grouped together. All the photosynthetic pigments in this cluster underwent noteworthy decline in cold–stressed seedlings (CS), in contrast to C or C−SNP. In CS−SNP plants, however, an increasing trend was observed. The third cluster included antioxidant enzyme variables (CAT, POD and APX), together with TPC, Car, PC, TSS and DPPH. The third cluster exhibited either a decreasing or unchanged pattern in C or C−SNP as to CS−SNP and CS. Additionally, a significant upward trend could be observed in CS−SNP in comparison with CS. The fourth cluster displayed variables related to membrane stability parameters such as EL, MDA and H_2O_2 content. Regarding the control groups (C and C−SNP), the parameters displayed considerable responses to CS versus CS−SNP.

Evaluation of Treatment–Variable Interaction Under −2 °C Based on Hierarchical Clustering and Heat Map Analysis

Hierarchical clustering resulted in the formation of four clusters based on the variables at −2 °C (Fig. 1c). The first cluster comprised PC, DPPH, APX, POD, TSS, TPC, Car, CAT, EL, MDA and H_2O_2. The heat map displayed a noticeable increase trends in all respective parameters under CS and CS−SNP in comparison with C or C−SNP. Applying exogenous NO to seedlings exposed to cold stress (CS–SNP) led to an increase in some parameters values (PC, DPPH, APX, POD, TSS, TPC, Car, CAT) compared to those subjected to CS alone. Nevertheless, parameters including H_2O_2, MDA and EL demonstrated a reduction upon NO application in relation to CS. The variable, Chlb, represented the second cluster which remained unchanged under CS conditions in both C and C–SNP. Chlb exhibited an increase responsiveness to NO application during cold stress (CS−SNP). The parameters RWC, FC, Ci, Gs, Pn and E were classified into the third cluster. In the case of seedling not exposed to cold stress, all parameters indicated a significant declining trend under CS and CS−SNP. However, the application of exogenous NO resulted in the upregulation of parameters in this cluster. The forth cluster consisted of TP and Chla.

Correlation Analysis and Principle Component Analysis (PCA) Between Treatment Groups and Variables Under Normal and Stress Conditions

Correlation analysis and PCA analysis were conducted to discover the correlation between various variables and treatment groups, namely C, C−SNP, CS and CS−SNP for each levels of cold treatment (Figs. 1b, d, 2a, b).

Evaluation of Treatment Groups and Variables Under 0 °C According to Correlation Analysis and PCA

The first two principal components (PC1 and PC2) explained 77.6% of total variance (Fig. 1b). PC1 had positive association with TPC, EL, Car, APX, Car, DPPH, TSS, PC, POD, H_2O_2 and MDA, and was negatively correlated with RWC, E, Ci, Pn, FC and Gs. Chla, Chlb and TP showed a positive correlation with PC2 (Table 1). Notably, strong overlap was observed between control groups (C and C−SNP) as anticipated (Fig. 1b). The scatter plot indicates a clear differentiation with minimal overlap between CS and C−SNP treatments. The results suggest that particular variables in the first and second clusters are strongly associated with the C and C−SNP treatments, respectively. Additionally, the variables in the third and fourth cluster are closely related to the CS and CS−SNP treatments, respectively. Among the 20 parameters measured in PCs, Car, TPC, EL accounted for the maximum variation, while the minimum variation was observed in the measured values of Gs, MDA and Ci (Fig. 1b).

Evaluation of Treatment Groups and Variables Under −2 °C According to Correlation Analysis and PCA

As a result, two PC1 and PC2 defined 60.6% and 17.5% of total variation respectively (Fig. 1d). EL, H_2O_2, POD, TPC, TSS, DPPH, CAT, MDA, PC, APX and Car were positive contributing measured values for PC1. The positive contributing values for PC2 were TP, Chla and Chlb (Table 2). PCA plot did not revealed any distinguishable separation between controls groups, while CS and CS−SNP treatment were different from each other (Fig. 1d). The variables of first and second cluster were associated with CS and CS−SNP treatments, and third and fourth cluster were related to the C and C−SNP treatments. Figure 2b showed that maximum variation were characterized with TPC and EL and minimum variation were designated by APX, Chlb, Gs, MDA and PC.

EL electrolyte leakage, *RWC* relative water content, *Chla* chlorophyll a, *Chlb* chlorophyll b, *TP* total chlorophyll, *Car* carotenoids, *FC* flavonoid content, *PC* proline content, *TPC* total phenolic compound, *TSS* total soluble sugars, *DPPH* 2,2–diphenyl–1–picrylhydrazyl, *MDA* malondialdehyde, *CAT* catalase, *POD* peroxidase, *APX* ascorbate peroxidase,

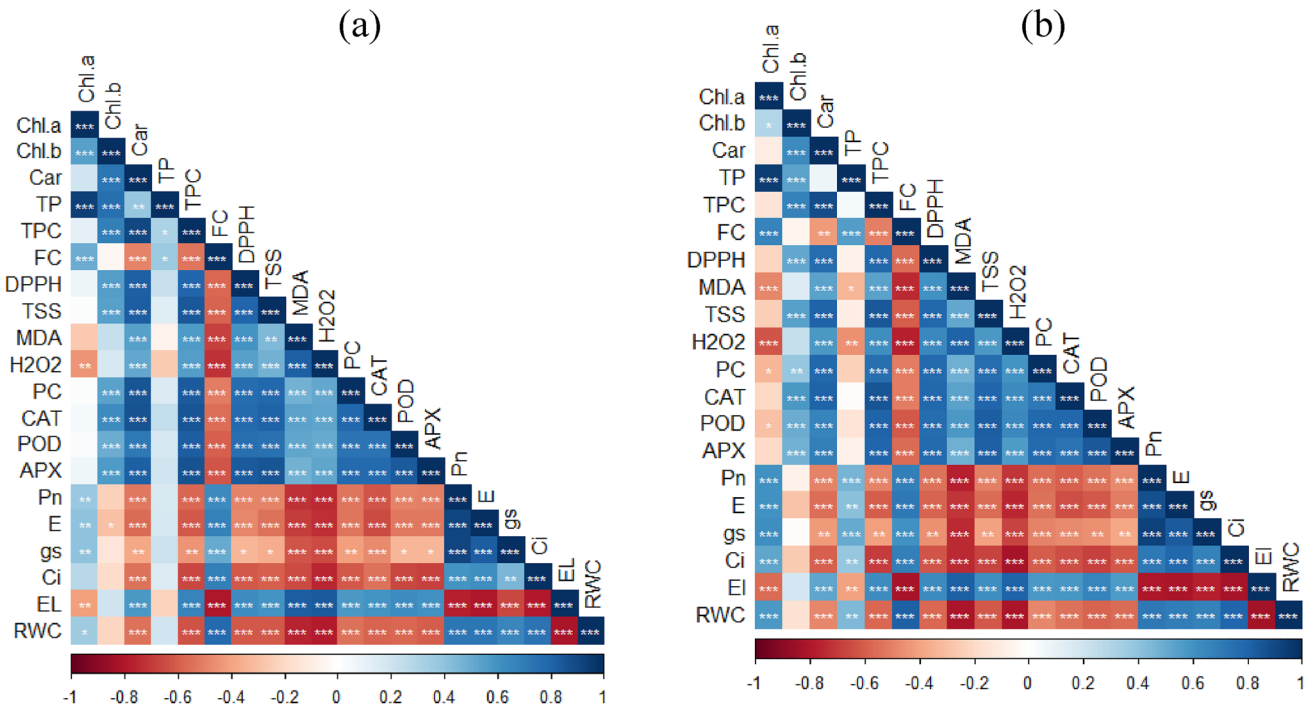

Fig. 2 Pearson correlation matrix for 21 quantitative variables (**a**) under 0 °C, **b** under –2 °C. *EL* electrolyte leakage, *RWC* relative water content, *Chla* chlorophyll a, *Chlb* chlorophyll b, *TP* total chlorophyll, *Car* carotenoids, *FC* flavonoid content, *PC* proline content, *TPC* total phenolic compound, *TSS* total soluble sugars, *DPPH* 2,2-diphenyl-1-picrylhydrazyl, *MDA* malondialdehyde, *CAT* catalase, *POD* peroxidase, *APX* ascorbate peroxidase, *Pn* net photosynthetic rate, *E* transpiration rate, *Gs* stomatal conductance, *Ci* intercellular CO_2 concentration, and H_2O_2 hydrogen peroxide

Pn net photosynthetic rate, *E* transpiration rate, *Gs* stomatal conductance, *Ci* intercellular CO_2 concentration, and H_2O_2 hydrogen peroxide.

EL electrolyte leakage, *RWC* relative water content, *Chla* chlorophyll a, *Chlb* chlorophyll b, *TP* total chlorophyll, *Car* carotenoids, *FC* flavonoid content, *PC* proline content, *TPC* total phenolic compound, *TSS* total soluble sugars, *DPPH* 2,2–diphenyl–1–picrylhydrazyl, *MDA* malondialdehyde, *CAT* catalase, *POD* peroxidase, *APX* ascorbate peroxidase, *Pn* net photosynthetic rate, *E* transpiration rate, *Gs* stomatal conductance, *Ci* intercellular CO_2 concentration, and H_2O_2 hydrogen peroxide.

Factor Analysis (FA) Based on Squared Cosines of Variables (SCV)

Evaluation of FA Under 0 °C

Of the tow PCs selected, PC1 possessed the highest R^2 value, followed by PC2. Therefore, PC1 has the most substantial data distribution. In PC1, the variable with the best data representation were TPC, EL, CAT, APX, Car, RWC, DPPH, TSS, PC, and E with corresponding maximum variation of SCV values of 0.79, 0.75, 0.73, 0.72, 0.71, 0.70, 0.69, 0.69, 0.67 and 0.67, respectively. Optimal data representation in PC2 can be observed for TP and Chla, with SCV values of 0.8 for both parameters.

Evaluation of FA Under –2 °C

The result displayed that in PC1, the variables with the best data representation were EL, Ci, H_2O_2, E, POD, PWC, TPC, TSS, DPPH, Pn, CAT and MDA with corresponding maximum SCV values of 0.78, 0.77, 0.76, 0.73, 0.70, 0.70, 0.69, 0.68, 0.68, 0.67, 0.67 and 0.67, respectively.

Stepwise Regression Analysis (SRA)

The SRA was carried out independently for two different conditions (0 and –2 °C). Tuber weight per plant (WTP) was defined as the function variable (Y), and the biochemical indicators derived from FA as independent variables.

In the 0 °C condition, among 12 characteristics, Chla, Car, TP, DPPH, APX and EL contributed to WPT (Tables 3 and 4). Car (6.30) and Chla (3.55) had the highest estimated coefficients (Table 4).

Table 1 Factor analysis, eigenvalue, total variance, and cumulative variance percentage for the 2 factor resulted from principal component analysis (PCA) under 0 °C

Variable	PC1	PC2
Chla	− 0.183	0.893
Chlb	0.497	0.731
Car	0.844	0.428
TP	0.031	0.898
TPC	0.891	0.3481
FC	− 0.778	0.380
DPPH	0.831	0.291
TSS	0.828	0.275
MDA	0.778	− 0.260
H_2O_2	0.798	− 0.385
PC	0.821	0.267
CAT	0.853	0.284
POD	0.814	0.244
APX	0.848	0.301
Pn	− 0.801	0.339
E	− 0.817	0.311
Gs	− 0.661	0.411
Ci	− 0.802	0.184
EL	0.869	− 0.333
RWC	− 0.834	0.295
Eigenvalue	11.63	3.88
Total variance %	58.17	19.40
Cumulative %	58.17	77.57

Table 2 Factor analysis, eigenvalue, total variance, and cumulative variance percentage for the 2 factor resulted from principal component analysis (PCA) under –2 °C

Variable	PC1	PC2
Chla	− 0.542	0.716
Chlb	0.389	0.768
Car	0.769	0.468
TP	− 0.363	0.806
TPC	0.828	0.465
FC	− 0.810	0.327
DPPH	0.822	0.327
TSS	0.816	− 0.236
MDA	0.827	0.361
H_2O_2	0.869	− 0.211
PC	0.794	0.247
CAT	0.819	0.372
POD	0.839	0.281
APX	0.784	0.355
Pn	− 0.820	0.335
E	− 0.854	0.220
Gs	− 0.751	0.413
Ci	− 0.875	0.122
EL	0.882	− 0.236
RWC	− 0.835	0.260
Eigenvalue	12.12	3.50
Total variance %	60.58	17.52
Cumulative %	60.58	78.11

The results of the SRA at –2 °C condition indicated that five characters, namely TPC, DPPH, TSS, Pn and EL, had significant effect on WTP (Table 5 and 6). TPC (2.70) with high coefficient value indicated the most important variable (Table 6).

As depicted in Figs. 1a and 2a, the foliar application of 50 µM SNP exogenously substantially enhanced the cold tolerance of potato plants that were exposed to LT. Nevertheless, specific varietal responses were noticed at 0 °C and –2 °C. Adato (1), SanteT (11), and Karelia (6) demonstrated the most exceptional levels of cold tolerance at 0 °C, while Donata (4) and Fontane (5) manifested the highest response to SNP at –2 °C. Adato (1), Croatia (3), and Karelia (6) displayed the lowest levels of cold tolerance at 0 °C. Furthermore, Croatia (3) exhibited the lowest response to SNP at –2 °C.

Discussion

Cold stress induces a number of physiological and metabolic changes, including changes in ROS, MDA, sucrose, lipid peroxides, proline, and others. PCA can therefore be

Table 3 ANOVA of stepwise regression model at 0 °C

Source	df	Sum of squares	Mean square	F Ratio	Probability
Model	6	510.54	85.09	13.42	< 0.0001*
Error	136	862.42	6.341		
Total	142	1372.96			

*Significant at the 0.01 probability level

Table 4 Parameter estimates stepwise selection linear regression model at 0 °C

Parameter	Estimate	Standard error	t Ratio	Prob > |t|
Intercept	5.983	2.845	2.10	0.0373*
Chla	20.582	5.806	3.55	0.0005*
Car	78.425	12.447	6.30	< 0.0001*
TP	− 20.063	5.341	− 3.76	0.0003*
DPPH	− 0.678	0.228	− 2.98	0.0034*
APX	− 0.108	0.040	− 2.68	0.0082*
EL	− 0.043	0.013	− 3.40	0.0009*

*Significant at the 0.01 probability level

TP total chlorophyll, *DPPH* 2,2-diphenyl-1-picrylhydrazyl, *APX* ascorbate peroxidase, *EL* electrolyte leakage

Table 5 ANOVA of stepwise regression model at − 2 °C

Source	df	Sum of square	Mean square	*F* ratio	Probability
Model	5	465.10	93.02	10.04	< 0.0001*
Error	138	1279.20	9.270		
Total	143	1744.30			

*Significant at the 0.01 probability level

Table 6 Parameter estimates stepwise selection linear regression model at − 2°C

Parameter	Estimate	Standard error	t Ratio	Prob > |t|
Intercept	15.70	1.911	8.21	< 0.0001*
TPC	0.095	0.035	2.70	0.0077*
DPPH	− 0.588	0.235	− 2.51	0.0134*
TSS	− 0.720	0.248	− 2.90	0.0043*
Pn	− 0.784	0.142	− 5.50	< 0.0001*
EL	− 0.052	0.014	− 3.67	0.0003*

TPC total phenolic compound, *DPPH* 2,2–diphenyl–1–picrylhydrazyl, *TSS* total soluble sugars, *Pn* net photosynthetic rate, *EL* electrolyte leakage

*Significant at the 0.01 probability level

considered a valuable tool to manage intricate data sets and identify treatment that elicit a wider range of responses (Kapoor et al. 2023; Balasubramanian and Praharaj 2023). A multitude of studies have employed statistical analysis techniques such as principal component analysis (PCA), heat maps, factor analysis (FA), structural equation modelling (SEM), and others to identify plant physiological parameters that can be used to detect stress effects (Ejaz et al. 2023; Kapoor et al. 2023; Balasubramanian and Praharaj 2023). In this research, EL, TPC, DPPH, CAT, E, and RWC exhibited considerable variation in PC1 at both low temperatures.

Cold stress has been discovered to result in oxidative harm in plants, causing cellular membrane injury, EL, and lipid peroxidation. EL serves as an indicator of membrane destruction caused by oxidative stress due to cold temperatures (Steponkus et al. 1990Y Murry et al. 1989). Similarly, MDA measurement, the ultimate outcome of lipid peroxidation, is frequently implemented as a gauge of cell membrane impairment amidst environmental stress (Shah et al. 2021). In this study, an increase in both MDA content and EL was noticeable in potato seedlings during exposure to chilling and freezing stress. However, the administration of NO significantly decreased cell damage compared to the control treatment CS−SNP (Figs. 1 and 2). This implies that treating with NO may alleviate the detrimental impacts of chilling stress on plant cells.

To manage oxidative stress, plants depend on antioxidant enzymes, such as SOD, POD, CAT, and APX, which are pivotal in controlling ROS and decreasing their toxicity (Zhang et al. 2017; Choudhury et al. 2017). In this study, the activity levels of CAT, APX and POD were found to be greater in CS and CS−SNP plants when compared to control plants under low temperature conditions (Fig. 1a and 2b). PCA analysis demonstrated a greater positive correlation between POD, APX, and CAT activity with CS-SNP treatment compared to CS treatment alone (Fig. 1b and 2b). This increase in activity could be attributed to NO stimulating the expression of antioxidant enzymes through MAPK and other signalling pathways (Neill et al. 2008). Furthermore, the treatment with CS showed a positive correlation with MDA, EL, and H_2O_2 (Fig. 1b and 2 b). Comparable affirmative impacts of exogenous NO have also been noticed in several other plant types, including rice (*Oryza sativa* L.), tea (*Camellia sinensis*) and melon (*Cucumis melo* L.) (Sohag, et al. 2020; Wang, et al. 2021a, b; Diao, et al. 2022).

Exposure to low temperatures in this study led to a decrease in the net photosynthetic rate (Pn) in CS and CS−SNP plants due to a drop in stomatal conductance (Gs), transpiration rate (E), and intercellular CO_2 concentration (Ci). However, the heat map data indicate that the CS plants exhibited much greater reductions in these parameters than the CS−SNP plants (Fig. 1 and 2). The cucumber (*Cucumis sativus* L.) crop also experienced similar negative effects due to reduced transpiration rates resulting from low temperatures (Zhang et al. 2020).

Cold stress can negatively impact the pigments involved in photosynthesis in plants (Zheng et al. 2021). Chl pigment content drastically decreased in both low temperatures in comparison to the controls (Fig. 1 and 2). Conversely, carotenoid content in the CS and CS−SNP treatments was higher than in the controls. A smaller reduction or even a slight increase in carotenoid content suggested the involvement of xanthophyll cycle carotenoids in dissipating thermal energy and safeguarding the reaction centers of Photosystem II (PSII) (Ding et al. 2017). Also, low temperature stress may lead to decrease in Chl biosynthesis in plants, partly due to the inhibition of 5−aminolevulinic acid biosynthesis (Allen and Ort 2001). Graziano and Lamattina (2007) have found that an elevation in Chl levels as result of NO can be linked to enhanced availability of iron, as NO consist of iron in its molecule. Iron plays a direct role in both electron transport chain reaction and Chl biosynthesis.

Under adverse environmental conditions, plants may undergo alterations in their intracellular osmotic potential, leading to cytoplasmic dehydration (Cheuvront and Kenefick 2014). To address with these challenges, plants have developed mechanisms to regulate osmotic balance by accumulating osmotic substances. When confronted with low temperature stress, plants utilize different strategies including the accumulation of soluble sugars, proline, flavonoids, and total phenols. These substances serve as osmoregulators, cryoprotectants, and signalling molecules. They help to maintain the osmotic balance, guard against the build-up of toxic substances generated by low temperature stress, and limit ice crystal formation due to water loss.

In this specific study, it was noted that the application of exogenous NO significantly increased the content of soluble sugars, proline, flavonoids, and total phenols in potato leaves. This study aligns with previous research indicating the beneficial effects of NO on the accumulation of osmoregulatory substances in plants (Dong et al. 2018).

Conclusions

The study's findings suggest that the application of NO proves to be an effective approach in mitigating the detrimental effects of LT stress on plants. NO treatments seem to possess positive effects as they can reduce the excessive accumulation of reactive oxygen species (ROS) caused by LT. This reduction is possibly achieved by enhancing the activities of enzymatic antioxidants, including CAT, APX, and POD, as well as non-enzymatic antioxidants such as DPPH and Car. Furthermore, the application of NO results in augmented levels of chlorophyll (Chl) and soluble solutes (such as PC, TPC, and TSS), alongside an enhancement in photosynthetic efficiency, when potato seedlings are exposed to LTs. Consequently, this study proposes that external NO application can provide a practical and effective technique to mitigate the adverse effects of LT stress.

Appendix

See Tables 7 and 8.

Table 7 Source of variation (mean square) for zero °C

Source of variation		Mean square																			
		Chl a	Chl b	Car	TP	TPC	FC	DPPH	MDA	TSS	H_2O_2	PC	CAT	POD	APX	Pn	E	Gs	Ci	EL	RWC
Genotype	11	0.936**	0.012**	0.003**	0.157**	172.24**	106.36**	2.604**	1.07*	18.79**	0.072**	0.021**	29,765**	1.34**	87.08**	11.03**	0.43**	0.062**	1451.2*	284**	136.36**
Temperature	1	0.102**	0.582**	0.102**	0.0005^{ns}	10,334.13**	4634.77**	239.37**	178.43**	1032.73**	11.37**	0.823**	2,321,163**	67.75**	8067.03**	423.49**	21.52**	0.915**	56,092.7**	54,262.2**	9923.11**
Treatment	1	0.655**	0.0432**	0.123**	0.486**	558.78**	225.28**	13.51**	38.10**	184.13**	2.411**	0.116**	104,909**	3.44**	852.35**	128.38**	4.04**	0.629**	2154.5^{ns}	6897.8**	919.72**
Genotype × Temperature	11	0.003^{ns}	0.0008**	0.0003**	0.003^{ns}	55.08**	24.33**	1.39**	0.947^{ns}	12.71^{ns}	0.079**	0.005**	16,638**	0.938**	44.56**	1.53^{ns}	0.113**	0.007**	158.6^{ns}	296.8**	88.94**
Genotype × Treatment	11	0.017*	0.0009**	0.001**	0.016^{ns}	30.04^{ns}	40.45**	0.837*	0.756^{ns}	2.66^{ns}	0.077**	0.002^{ns}	16,598**	0.481**	20.35^{ns}	4.36**	0.259**	0.006**	709.2^{ns}	257.6**	29.06**
Temperature × Treatment	1	0.007^{ns}	0.004**	0.002**	0.029^{ns}	86.28*	69.65**	9.27**	0.0978^{ns}	85.06**	0.852**	0.035**	89,264**	4.19**	283.14**	1.39^{ns}	0.195*	0.005*	1688.2^{ns}	2816.8**	688.35**
Genotype × Temperature × Treatment	11	0.002^{ns}	0.0004^{ns}	0.0001*	0.002^{ns}	5.23^{ns}	16.60**	1.46**	0.337^{ns}	4.28^{ns}	0.09**	0.002^{ns}	12,481**	0.116^{ns}	49.66**	1.7^{ns}	0.05^{ns}	0.004**	402.3^{ns}	51.4^{ns}	15.85*
Error	96	0.008	0.0003	0.00005	0.009	21.84	6.66	0.407	0.522	7.88	0.024	0.002	3426	0.209	11.82	1.32	0.43	0.001	677.2	104.4	7.86
Total	143																				

**, * and ns are significant at the 0.01 and 0.05 of probability level and non-significant, respectively

Table 8 Source of variation (mean square) for -2 °C

Source of variation		Chl a	Chl b	Car	TP	TPC	FC	DPPH	MDA	TSS	H_2O_2	PC	CAT	POD	APX	Pn	E	Gs	Ci	EL	RWC
Genotype	11	0.68**	0.01**	0.003**	0.116**	301.42**	106.12**	1.93**	34.9**	0.903^{ns}	0.212**	0.039**	35,895**	0.654**	56.61**	19.154**	0.811**	0.099*	8	1409.4**	412.2**	404.3**
Temperature	1	0.561**	0.042**	0.079**	0.223**	13,823.41**	4628.08**	313.76**	2675.73**	268.12**	31.66**	1.82**	2,353,335**	78.71**	5979.35**	608.65**	38.13**	1.71**		253,143.4**	83,446.8**	28,564.04**
Treatment	1	0.634**	0.038**	0.011**	0.494**	935.93**	290.59**	15.38**	388.65**	53.28**	3.69**	0.271**	88,458**	3.55**	860.03**	142.31**	5.75**	0.794**		11,590.3**	9588**	2868.78**
Genotype × Temperature	11	0.015**	0.002**	0.0003**	0.022**	66.84**	14.71*	3.002**	26.49*	1.13*	0.201**	0.017**	23,939**	0.438*	63.23**	6.87**	0.252**	0.016**		1522.4**	559.2**	164**
Genotype × Treatment	11	0.0123**	0.0007**	0.0002**	0.009^{ns}	22.36^{ns}	30.8**	1.03^{ns}	14.33^{ns}	1.1*	0.218**	0.009*	16,022**	0.484**	19.78*	4.13**	0.388**	0.009**		2007.7**	329.7*	104.57**
Temperature × Treatment	1	0.0054^{ns}	0.002**	0.002**	0.014^{ns}	200.45**	112.11**	10.19**	226.73**	0.773^{ns}	0.994**	0.098**	76,405**	4.19**	356.31**	1.48**	0.125*	0.001**		8919.3**	3626.6**	2073.04**
Genotype × Temperature × Treatment	11	0.0021^{ns}	0.0003*	0.0001^{ns}	0.003^{ns}	18.97^{ns}	16.71**	1.46**	2.86^{ns}	0.496^{ns}	0.131**	0.011**	13,810**	0.155^{ns}	37.37**	2.01**	0.076**	0.004**		1698.9**	68.2^{ns}	47.68*
Error	96	0.006	0.0002	0.00008	0.006	19.00	7.43	0.635	12.83	0.538	0.05	0.005	3243	0.205	9.2	0.211	0.025	0.0002		552.6	152.1	24.86
Total	143																					

**, * and ns are significant at the 0.01 and 0.05 of probability level and non-significant, respectively

Declarations

Conflict of interest The authors declare no conflict of interest.

References

Abdi H, Williams LJ (2010) Principal component analysis. Wiley Interdisc Rev 2(4):433–459

Abe N, Murata T, Hirota A (1998) Novel 1,1-diphenyl-2-picryhy-drazyl-radical scavengers, bisorbicillin and demethyltrichodimerol, from a fungus. Biosci Biotechnol Biochem 62(4):61–662. https://doi.org/10.1271/bbb.62.661

Anelia GD (2017) Signaling molecules in plants: exogenous application. Acta Sci Agric 1:38–41

Balasubramanian P, Praharaj PT (2023) Principal component analysis revealed the key influencing factors of kombucha bacterial cellulose yield and productivity. Bioresource Technol Rep 23:101539. https://doi.org/10.1016/j.biteb.2023.101539

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39(1):205–207. https://doi.org/10.1007/BF00018060

Chang CC, Yang MH, Wen HM, Chern JC (2002) Estimation of total flavonoid content in propolis by two complementary colometric methods. J Food Drug Anal 10(3):178–182. https://doi.org/10.38212/2224-6614.2748

Chen HH, Li PH (1980) Characteristics of cold acclimation and deacclimation in tuber-bearing Solanum species. Plant Physiol 65(6):1146–1148. https://doi.org/10.1104/pp.65.6.1146

Cheuvront SN, Kenefick RW (2014) Dehydration: physiology, assessment, and performance effects. Compr Physiol 4(1):257–285. https://doi.org/10.1002/cphy.c130017

Choudhury FK, Rivero RM, Blumwald E, Mittler R (2017) Reactive oxygen species, abiotic stress and stress combination. Plant J 90(5):856–867. https://doi.org/10.1111/tpj.13299

Dere S, Gines T, Sivaci R (1998) Spectrophotometric determination of chlorophyll-a, b and total carotenoid contents of some algae species using different solvents. Turk J Bot 22(1):13–17

Diao QN, Cao YY, Wang H, Zhang YP, Shen HB (2022) Nitric oxide confers chilling stress tolerance by regulating carbohydrate metabolism and the antioxidant defense system in melon (*Cucumis melo* L.) seedlings. Hortscience 57(10):1249–1256. https://doi.org/10.21273/HORTSCI16677-22

Ding F, Wang M, Liu B, Zhang S (2017) Exogenous melatonin mitigates photoinhibition by accelerating non-photochemical quenching in tomato seedlings exposed to moderate light during chilling. Front Plant Sci 8:244. https://doi.org/10.3389/fpls.2017.00244

Ding Y, Shi Y, Yang S (2020) Molecular regulation of plant responses to environmental temperatures. Mol Plant 13(4):544–564. https://doi.org/10.1016/j.molp.2020.02.004

Dong N, Li Y, Qi J, Chen Y, Hao Y (2018) Nitric oxide synthase-dependent nitric oxide production enhances chilling tolerance of walnut shoots in vitro via involvement chlorophyll fluorescence and other physiological parameter levels. Sci Hortic 230:68–77. https://doi.org/10.1016/j.scienta.2017.110.016

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith FAJN (1951) A colorimetric method for the determination of sugars. Nature 168(4265):167. https://doi.org/10.1038/168167a0

Ejaz I, Pu X, Naseer MA, Bohoussou YND, Liu Y, Farooq M, Zhang J, Zhang Y, Wang Z, Sun Z (2023) Cold and drought stresses in wheat: a global meta-analysis of 21st century. J Plant Growth Regul 42(9):5379–5395. https://doi.org/10.1007/s00344-023-10960-x

Esim N, Atici O (2014) Nitric oxide improves chilling tolerance of maze by affecting apoplastic antioxidative enzyme in leaves. Plant Growth Regul 72:29–38. https://doi.org/10.1007/s10725-013-9833-4

Fan H, Du C, Xu Y, Wu X (2014) Exogenous nitric oxide improves chilling tolerance of Chinese cabbage seedlings by affecting antioxidant enzymes in leaves. Hortic Environ Biotechnol 55:159–165. https://doi.org/10.1007/s13580-014-0161-z

Fan J, Chen K, Amombo E, Hu Z, Chen L, Fu J (2015) Physiological and molecular mechanism of nitric oxide (NO) involved in bermudagrass response to cold stress. PLoS ONE 10(7):e0132991. https://doi.org/10.1371/journal.pone.0132991

Fancy NN, Bahlmann AK, Loake GJ (2017) Nitric oxide function in plant abiotic stress. Plant Cell Environ 40(4):462–472

Graziano M, Lamattina L (2007) Nitric oxide accumulation is required for molecular and physiological responses to iron deficiency in tomato roots. Plant J 52(5):949–960 https://doi.org/10.1111/j.1365-313X.2007.03283.x

Hussain HA, Hussain S, Khaliq A, Ashraf U, Anjum SA, Men S, Wang L (2018) Chilling and drought stresses in crop plants: implications, cross talk, and potential management opportunities. Front Plant Sci 9:393. https://doi.org/10.3389/fpls.2018.00393

Kapoor A, Krishnamoorthy N, Pathy A, Balasubramanian P (2023) Chemometric analysis unravelling the effect of key influencing factors on biochar yield. Algal Res 69:102908. https://doi.org/10.1016/j.algal.2022.102908

Li F, Bian CS, Xu JF, Pang WF, Liu J, Duan SG, Lei ZG, Jiwan P, Jin LP (2015) Cloning and functional characterization of SAD genes in potato. PLoS ONE 10(3):e0122036. https://doi.org/10.1371/journal.pone.0122036

Li Y, Zhang Q, Qu L, Ji D, Liu T, Lan R, Li X, Jin L (2020) Response to the cold stress signaling of the tea plant (*Camellia sinensis*) elicited by chitosan oligosaccharide. Agronomy 10(6):915. https://doi.org/10.3390/agronomy10060915

Liu T, Xu JJ, Li JM, Hu X (2019) NO is involved in JA- and H2O2-mediated ALA-induced oxidative stress tolerance at low temperatures in tomato. Environ Exp Bot 161:334–343. https://doi.org/10.1016/j.envexpbot.2018.10.020

Liu X, Wang L, Liu L, Guo Y, Ren H (2011) Alleviating effect of exogenous nitric oxide in cucumber seedling against chilling stress. Afr J Biotech 10(21):4380–4386. https://doi.org/10.5897/AJB10.812

Longo V, Janmohammadi M, Zolla L, Rinalducci S (2018) What can small molecules tell us about cold stress tolerance in plants? Cold Toler Plants. https://doi.org/10.1007/978-3-030-01415-5_8

Mao H, Chen M, Su Y, Wu N, Yuan M, Yuan S, Brestic M, Zivcak M, Zhang H, Chen Y (2018) Comparison on photosynthesis and antioxidant defense systems in wheat with different ploidy levels and octoploid triticale. Int J Mol Sci 19(10):3006. https://doi.org/10.3390/ijms19103006

Mora-Herrera ME, Lopez-Delgado HA (2007) Freezing tolerance and antioxidant activity in potato microplants induced by abscisic acid treatment. Am J Potato Res 84:467–475. https://doi.org/10.1007/BF02987883

Murray MB, Cape JN, Fowler D (1989) Quantification of frost damage in plant tissues by rates of electrolyte leakage. New Phytol 113(3):307–311. https://doi.org/10.1111/j.1469-8137.1989.tb02408.x

Nabi RBS, Tayade R, Hussain A, Kulkarni KP, Imran QM, Mun BG, Yun BW (2019) Nitric oxide regulates plant responses to drought, salinity, and heavy metal stress. Environ Exp Bot 161:120–133. https://doi.org/10.1016/j.envexpbot.2019.02.003

Neill S, Barros R, Bright J, Desikan R, Hancock J, Harrison J, Morris P, Ribeiro D, Wilson I (2008) Nitric oxide, stomatal closure, and abiotic stress. J Exp Bot 59(2):165–176. https://doi.org/10.1093/jxb/erm293

Palta JP, Bamberg JB, Chen YK, Vega SE, Weiss LS, Karlsson BH (1997) Understanding genetic control of freezing resistance using potato species as a model system. Plant Cold Hardiness. https://doi.org/10.1007/978-1-4899-0277-1_7

Palta JP, Li PH (1979) Forest-Hardiness in Relation to Leaf Anatomy and Natural Distribution of Several Solanum Species 1. Crop Sci 19(5):665–671. https://doi.org/10.2135/cropsci1979.0011183X001900050031x

Palta JP, Whitaker BD, Weiss LS (1993) Plasma membrane lipids associated with genetic variability in freezing tolerance and cold acclimation of Solanum species. Plant Physiol 103(3):793–803. https://doi.org/10.1104/pp.103.3.793

Rensink WA, Iobst S, Hart A, Stegalkina S, Liu J, Buell CR (2005) Gene expression profiling of potato responses to cold, heat, and salt stress. Funct Integr Genomics 5:201–207. https://doi.org/10.1007/s10142-005-0141-6

Sánchez-Vicente I, Fernández-Espinosa MG, Lorenzo O (2019) Nitric oxide molecular targets: reprogramming plant development upon stress. J Exp Bot 70(17):4441–4460. https://doi.org/10.1093/jxb/erz339

Sergiev I, Alexieva V, Karanov E (1997) Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plants. C R Acad Bulg Sci 51(3):121–124. https://doi.org/10.1046/j.1365-3040.2001.00778.x

Shah W, Ullah S, Ali S, Idrees M, Khan MN, Ali K, Khan A, Ali M, Younas F (2021) Effect of exogenous alpha-tocopherol on physio-biochemical attributes and agronomic performance of lentil (*Lens culinaris* Medik.) under drought stress. PLoS ONE 16(8):200. https://doi.org/10.1371/journal.pone.0248200

Sharp RE, Hsiao TC, Silk WK (1990) Growth of the maize primary root at low water potentials: II. Role of growth and deposition of hexose and potassium in osmotic adjustment. Plant Physiol 93(4):1337–1346. https://doi.org/10.1104/pp.93.4.1337

Shi C, Yang F, Liu Z, Li Y, Di X, Wang J, Lin J (2021) Uniform water potential induced by salt, alkali, and drought stresses has different impacts on the seedling of Hordeum jubatum: from growth, photosynthesis, and chlorophyll fluorescence. Front Plant Sci 12:733236. https://doi.org/10.3389/fpls.2021.733236

Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 16(3):144–158. https://doi.org/10.5344/ajev.1965.16.3.144

Sohag AAM, Tahjib-Ul-Arif M, Afrin S, Khan MK, Hannan MA, Skalicky M, Mortuza MG, Brestic M, Hossain MA, Murata Y (2020) Insights into nitric oxide-mediated water balance, antioxidant defence and mineral homeostasis in rice (*Oryza sativa* L.) under chilling stress. Nitric Oxide 100:7–16. https://doi.org/10.1016/j.niox.2020.04.001

Steponkus PL, Lynch DV, Uemura M (1990) The influence of cold acclimation on the lipid composition and cryobeheviour of the plasma membrane of isolated rye protoplasts. Philos Trans R Soc Lond B 326(1237):571–583. https://doi.org/10.1098/rstb.1990.0032

Wang A, Li J, Al-Huqail AA, Al-Harbi MS, Ali EF, Wang J, Ding Z, Rekaby SA, Ghoneim AM, Eissa MA (2021a) Mechanisms of chitosan nanoparticles in the regulation of cold stress resistance in banana plants. Nanomaterials 11(10):2670. https://doi.org/10.3390/nano11102670

Wang Y, Yu Q, Li Y, Li J, Chen J, Liu Z, Huang JA, Al-Harbi MS, Ali EF, Eissa MA (2021b) Mechanisms of nitric oxide in the regulation of chilling stress tolerance in *Camellia sinensis*. Horticulturae 7(10):410. https://doi.org/10.3390/horticulturae7100410

Wu P, Kong Q, Bian J, Ahmmed GJ, Gui H, Xu W, Yang Z, Cui J, Liu H (2022) Unveiling molecular mechanisms of nitric oxide-induced low-temperature tolerance in cucumber by transcriptome profiling. Int J Mol Sci 23(10):5615. https://doi.org/10.3390/ijms23105615

Yan C, Zhang N, Wang Q, Fu Y, Wang F, Su Y, Xue B, Zhou L, Liao H (2021) The effect of low temperature stress on the leaves and microRNA expression of potato seedlings. Front Ecol Evol 9:727081. https://doi.org/10.3389/fevo.2021.727081

Zhang L, Guo X, Zhang Z, Wang A, Zhu J (2021) Cold-regulated gene *LeCOR413PM2* confers cold stress tolerance in tomato plants. Gene 764:145097. https://doi.org/10.1016/j.gene.2020.145097

Zhang T, Che F, Zhang H, Pan Y, Xu M, Ban Q, Han Y, Rao J (2017) Effect of nitric oxide treatment on chilling injury, antioxidant enzymes and expression of *CmCBF1* and *CmCBF3* genes in cold-stored Hami- melon (*Cucumis melo* L.) fruit. Postharvest Biol Technol 127:88–98. https://doi.org/10.1016/j.postharvbio.2017.01.005

Zheng S, Su M, Wang L, Zhang T, Wang J, Xie H, Wu X, Haq SIU, Qiu QS (2021) Small signaling molecules in plant response to cold stress. J Plant Physiol 266:153534. https://doi.org/10.1016/j.jplph.2021.153534

Zhang Z, Wu P, Zhang W, Yang Z, Liu H, Ahammed GJ, Cui J (2020) Calcium is involved in exogenous NO-induced enhancement of photosynthesis in cucumber (Cucumis sativus L.) seedlings under low temperature. Sci Hortic 261:108953. https://doi.org/10.1016/j.scienta.2019.108953