Phyton-
International Journal of
Experimental Botany

Tech Science Press

DOI: 10.32604/phyton.2022.018708

ARTICLE

Nitric Oxide Alleviates Photochemical Damage Induced by Cadmium Stress in Pea Seedlings

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Received: 12 August 2021 Accepted: 18 October 2021

ABSTRACT

Cadmium (Cd), a life threatening hazardous heavy metal is abundant in nature. Cd amounts are greater in leaves than other plant parts, and it shows considerable effects on photosynthesis. Nitric oxide (NO), a free radical present in living organisms, is now known as an important signaling molecule playing various physiological processes in plants. In this study, the possible ameliorative effect of NO on photosynthesis was examined on pea seedlings grown under Cd stress. Results showed that chlorophyll, net photosynthetic rate, transpiration rate, stomatal conductance, photochemical efficiency of Photosystem II and Photosystem I decreased, and Fo and non-photochemical parameters for PSII and PSI significantly increased due to Cd stress. This suggests that Cd affects the photochemistry efficiency at both the PSII and PSI levels. Nitric oxide supplementation through SNP ameliorated Cd stress by enhancing all the above mentioned parameters but causing a reduction in the Fo, and non-photochemical parameters of PSII and PSI in pea plants. These data indicate that the exogenous application of NO was useful in mitigating Cd-induced damage to photosynthesis in pea seedling.

KEYWORDS

Chlorophyll; fluorescence; oxidative stress; Photosystem I; Photosystem II

1 Introduction

Heavy metals including Cd occur naturally in soils in trace amounts. Anthropogenic activities have led to Cd contamination of agricultural lands [1]. Cd is taken up from soil and transported to all parts of plant, as a result become potential hazard for both plant and animals [2]. Cd causes (1) inhibition of many physiological processes in plants like nitrogen assimilation, mineral nutrition, photosynthesis, respiration, transpiration, and carbohydrate metabolism (2) increases in chlorosis, wilting, necrotic lesions, and oxidative stress, and induction of senescence, all of which reduce biomass production [3,4]. Furthermore, Cd stress has also been associated with reactive oxygen species (ROS) generation, including superoxide ions ($O_2^{-\bullet}$), hydroxyl radicals ($HO^{\bullet}$) and hydrogen peroxide (H_2O_2) [5,6].

Photosynthesis, one of the most important physiological processes in plants, affects the entire metabolism of plants. Cd (1) disrupts thylakoid and chloroplast of the photosynthetic apparatus [1,7,8], (2) decreases Chl and carotenoid pigments [9], (3) inhibits the enzymes involved in Chlorophyll synthesis [10] in addition to RUBISCO [11,12], and (4) affects photoreduction and association of protochlorophyllide in etioplast inner membrane preparations and dark-grown leaves of wheat. Chlorophyll biosynthesis is a physiological phenomenon which is connected to photosynthetic efficiency of plants. The δ-amino levulinic acid, an important intermediate of the chlorophyll biosynthetic pathway, can be synthesized from 5-carbon compounds, like glutamate [12]. Further, the enzyme δ-amino levulinic acid dehydratase, of the chlorophyll biosynthetic pathway, has been shown to be hindered by Cd in radish leaves. Cd^{2+} is also reported to replace the Mg^{2+} central atom of chlorophyll in water plants [13,14].

PSII plays an imperative role in the response to environmental worries and stresses in photosynthesis in higher plants [15]. It was reported that Cd targets PSII instead of PSI [16,17]. The NADP oxidoreductase, ATP-synthase and oxygen-evolving complex of the photosynthetic electron transport chain are considered sensitive to Cd [18]. Oxidizing or reducing sites of PSII are probably common sites for heavy metal action in plants [13]. Cd^{2+} replaces Mn^{2+} from the water-oxidizing system of PSII leading to PSII reaction inhibition [7]. Only few studies have been done to study the effects of Cd on PSI electron transport *in vivo*. Most of the effects of Cd on the photosynthetic electron transport have been shown *in vitro*. PAM fluorometry and especially P700 absorbance–two noninvasive, net photosynthetically informative measuring techniques–are really important tools to detect photochemical changes with appreciable sensitivity due to heavy metal toxicity in photosynthetic organisms. Moreover, P700 absorbance measurements may well provide additional *in vivo* data to magnify our information on the impacts of heavy metals on PSI photochemistry in plants.

NO is one among the few known gaseous signaling molecules [17]. The high reactivity and diffusibility make NO perfect for a transient signaling molecule [19–21]. Both photorespiration and photosynthesis can be influenced by NO in various plants. Depending on its concentrations, the plant age or tissue, and the form of stress, some researchers reported NO as a stress promoting factor, whereas others have shown its ameliorating role [22–24]. Treatments with NO donors, such as SNP, have been shown to enhance photosynthetic rate, chlorophyll concentration, stomatal conductance and transpiration rate in plants [25]. In contrast, SNP diminished the quantity of β subunits of the RUBISCO subunit-binding protein and Rubisco activase in mung bean [26]. Cd toxicity is a major problem which inhibits many physiological processes in plants including photosynthesis and thus reduces crop yield. Therefore, the present work was undertaken with the objective of studying the ameliorating role of NO in alleviating photochemical damage induced by Cd stress in pea.

2 Materials and Methods

2.1 Plant Material, Treatments and Growing Conditions

Pea (*Pisum sativum* L.) seeds were surface sterilized by immersion in a 0.5% sodium hypochlorite (NaOCl) solution for 5 min and then washed three times with sterile, distilled water. Seeds were germinated on filter paper moistened with deionized water for 4 days. Following germination, seedlings were transferred to plastic pots filled with 2L hoagland solution [27] as a control treatment. At the same time, two concentrations of $CdCl_2$ (50 and 200 μM) with or without SNP (NO donor) (50 μM) were added to the nutrient solution and used as the treatment solutions. Seedlings were grown in a growth chamber with 12 h day/12 h night, temperatures of 25/20°C day/night, white fluorescent light intensity of 350 μmol photons $m^{-2}{\cdot}s^{-1}$, and 70% relative humidity. Growth solutions were continuously aerated and renewed every three days. After 15 days of treatment, a full expanded leaf was used for photosynthetic parameters measurements.

2.2 Determination of Chlorophyll

The chlorophyll determination was made following Arnon [28]. After discarding major veins and any tough, fibrous tissue, leaves were cut in small pieces. Fresh leaves weighing 0.5 g were grinded in 10 ml of 80% acetone (acetone:water 80:20 v:v) using a mortar and pestle. Leaf homogenate was filtered using filter paper. The extract was transferred to a graduated tube and filled to 10 ml with 80% acetone and assayed immediately. The absorbance was measured at 664, 647 and 470 nm in 1 cm cells using an 80% aqueous acetone as blank. Chl a, Chl b, total Chl (Chl a + b) and carotenoids concentrations were calculated using Arnon equation and expressed as $mg \cdot g^{-1}$ fresh weight.

2.3 Determination of Photosynthetic Rate, Transpiration Rate, Stomatal Conductance and Water Use Efficiency

Gas exchange was measured with a portable photosynthesis system CI-340 (CID Bio-Science). Photosynthetic (Pn) (μmol CO_2 $m^{-2} \cdot s^{-1}$), transpiration (Tn) (mmol H_2O m^{-2} s^{-1}), and stomatal conductance rates (mmol $m^{-2} \cdot s^{-1}$) were registered every 30 sec on fully expanded leaves during 40 min. Measurements were performed on one randomly selected seedling per pot. From these data, a daily mean of measured indices was calculated. Water use efficiency (WUE) (μmol CO_2 mmol H_2O^{-1}) was calculated by dividing the photosynthetic rate by the transpiration rate. The environmental conditions during the experiments were as follows: air flow rate 400 $\mu mol \cdot s^{-1}$, block and leaf temperatures 25°C, CO_2 concentration in sample cell 300–400 μmol CO_2 mol^{-1}, relative humidity in sample cell 30%, lightness in quantum 180 μmol $m^{-2} \cdot s^{-1}$.

2.4 Measurement of Chlorophyll Fluorescence and P700 Parameter

Chlorophyll fluorescence parameters and the redox change of P700 were assessed with a Dual-PAM-100 measuring system (Walz) on fully expanded leaves. Leaves of the different treatments (Control, 50 μM SNP, 50 μM Cd, 50 μM Cd + 50 μM SNP, 200 μM Cd, and 200 μM Cd + 50 μM SNP) were exposed to darkness for 30 min before determining the following Chlorophyll fluorescence parameters: *Fo*; *Fm*; *Fv*/*Fm* = (*Fm*-*Fo*)/*Fm*; qN = Fm-Fm'/Fm-Fo; qL = qP(Fo'/F'); qP = (Fm'-F)/(Fm'-Fo'); NPQ = (Fm-Fm')/(Fm'); Y(II) = (Fm'-Fo')/Fm'; D = Fo′/Fm′; Y(NPQ) = 1-Y-Y(NO); Y(NO) = 1/NPQ + 1 + qL((Fm/Fo)-1); Y(I) = 1-Y(ND)-Y(NA); Y(NA) = (Pm-Pm')/Pm, and Y(ND) = 1-P700 red.

2.5 Statistical Analysis

One-way Analysis of Variance (ANOVA) was carried out using Graph Pad PRISM version 5.01. Multiple linear regression (MLR) and β-regression analysis were conducted using Microsoft Excel 2010 [29,30]. Values shown are means ±1 standard error (SE), and *, ** and *** represent significant differences at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

3 Results

3.1 SNP Enhances Chlorophyll and Carotenoid Concentration under Cd Stress

Total Chl and carotenoids decreased by 3.95% and 24.00%, respectively, at 50 μM Cd, and by 20.00% and 46.00%, respectively, at 200 μM Cd-treated seedlings compared to controls (Figs. 1A and 1B). Ratios of Chl a/b and Chl/Carotenoid increased by 6.00% and 24.00%, respectively, at 50 μM Cd, and 14.00% and 46.00%, respectively, at the 200 μM Cd treatment compared to controls (Figs. 1C and 1D). Application of 50 μM SNP alone as well as in combination with 50 or 200 μM Cd improved photosynthetic pigment concentrations in Cd stressed plants. Application of 50 μM SNP with 50 or 200 μM Cd led to 15.00% or 22.00% increase in chlorophyll concentration, respectively; 13.00% or 50.00% increase in carotenoid concentration, respectively, 19.00% or 21.00% decrease in chl-a/b ratio, respectively, and 2.00% increase or 17.00% decrease in chl/carotenoids ratio, respectively, compared to the plants treated with 50 or

200 μM Cd alone. Moreover, multiple linear regression analysis (MLR) revealed that seedlings in presence of Cd resulted in a reduction of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid concentrations. However, the application of nitric oxide donor (SNP) along with Cd significantly increased the concentrations of all pigments. This is, while the relationships between Cd *vs.* the pigment concentrations were negative, those between Cd + SNP *vs*. the pigment concentrations were positive (Table 1).

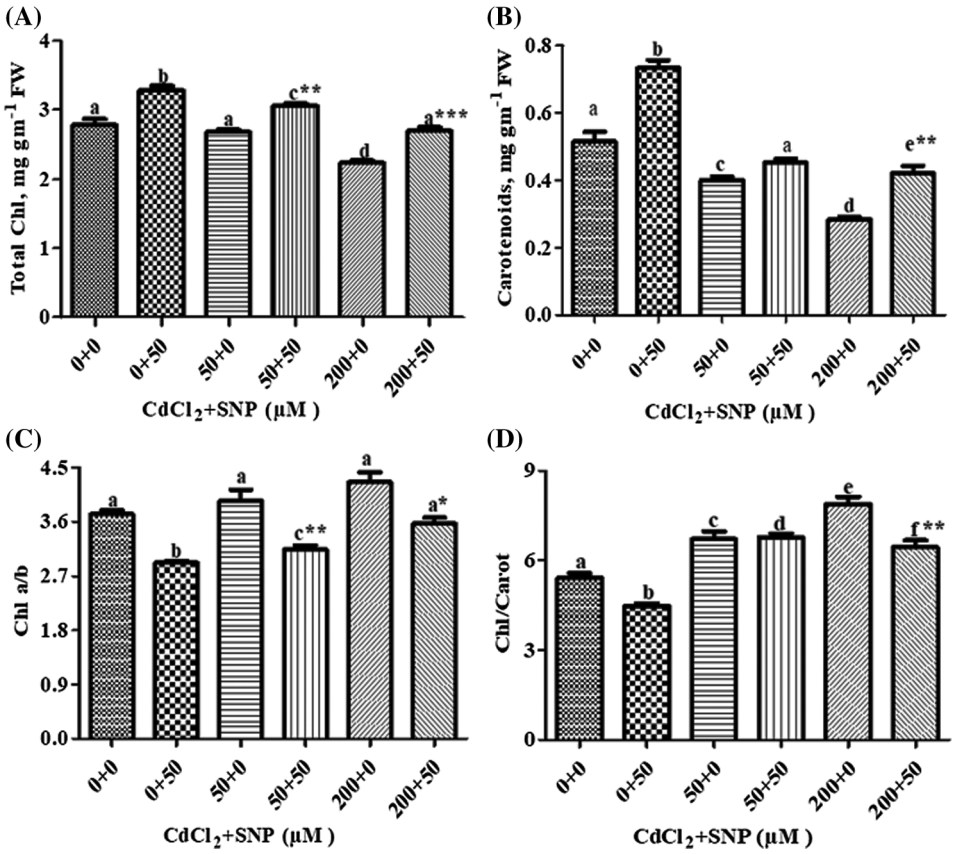

Figure 1: Effect of Cd, SNP and their combinations on total chlorophyll (A), carotenoids (B), chlorophyll a/b (C) and chlorophyll/carotenoid (D) In leaves of pea seedlings. Values are means ±1 standard error (SE). Different letters represent significant differences compared to the control treatment at $P \leq 0.05$. *, ** and *** represent significant differences between the Cd + SNP treatment and the Cd treatment at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively

Table 1: Multiple linear regression analysis showing the effect of SNP (50 μM) on 50 or 200 μM $CdCl_2$ induced-changes on pigment concentrations and photosynthetic parameters, PSII and PSI in leaves of pea seedlings

S. No.	MLR equation	β-regression coefficient		R
		β_1	β_2	
1	Total Chl (mg·g^{-1} FW) = 2.1837 − 0.0047 X1 + 0.0148 X2	−0.7277	0.6649	0.9857**
2	Carotenoids (mg·g^{-1} FW) = 5.1104 + 0.061 X1 + 0.0268 X2	−0.5881	0.7590	0.9602**

(Continued)

Table 1 (continued)

S. No.	MLR equation	β-regression coefficient		R
		β_1	β_2	
3	Pn (μ mol $m^{-2}{\cdot}s^{-1}$) = 5.9723 – 0.0170 X1 + 0.0584 X2	−0.6490	0.6531	0.9207*
4	Et (m mol $m^{-2}{\cdot}s^{-1}$) = 4.1872 – 0.01609 X1 + 0.002 X2	−0.7969	0.0348	0.7976
5	Gs (m mol $m^{-2}{\cdot}s^{-1}$) = 103.8919 – 0.3419 X1 + 0.0440 X2	−0.8054	0.0305	0.8060
6	Fo = 1.5592 + 0.0016 X1 – 0.0140 X2	0.3709	−0.9160	0.9882**
7	Fm = 5.4495 – 0.0088 X1 + 0.0084 X2	−0.7619	0.2141	0.7914
8	Fv/Fm = 0.6915 – 0.0010 X1 + 0.0039 X2	−0.6214	0.7215	0.9522*
9	qN = 0.4085 + 0.0017 X1 – 0.0043 X2	0.7096	−0.5288	0.8850*
10	qL = 0.4703 + 0.0001 X1 – 0.0012 X2	0.0645	−0.1434	0.1573
11	qP = 0.8444 – 0.0017 X1 + 0.0057 X2	−0.5946	0.5670	0.8216
12	NPQ = 0.3638 + 0.0010 X1 – 0.0018 X2	0.7594	−0.3902	0.8538
13	Y(II) = 0.5644 – 0.0018 X1 + 0.0064 X2	−0.6480	0.6712	0.9329*
14	D = 0.3733 + 0.0012 X1 – 0.0046 X2	0.6401	−0.7022	0.9501*
15	Y(NPQ) = 0.1175 + 0.0008 X1 – 0.0023 X2	0.6992	−0.5682	0.9009*
16	Y(NO) = 0.3179 + 0.0009 X1 – 0.0041 X2	0.5996	−0.7358	0.9492*
17	Y(I) = 0.3845 – 0.0011 X1 + 0.0002 X2	−0.9416	0.0515	0.9430*
18	Y(NA) = 0.1666 – 0.0001 X1 – 0.0008 X2	−0.1753	−0.4184	0.4536
19	Y(ND) = 0.4054 + 0.0014 X1 + 0.0003 X2	0.9715	0.0645	0.9736**

Notes: X1 = $CdCl_2$ (μM), X2 = sodium nitroprusside (μM), beta regression coefficients, β1 = $CdCl_2$, β2 = SNP, R = multiple correlation coefficient, Pn = net photosynthetic rate, Et = transpiration rate, Gs = stomatal conductance, Fo = dark fluorescence yield, Fm = maximal fluorescence yield, Fv/Fm = maximal PSII quantum yield, qN = coefficient of non-photochemical quenching, qL = coefficient of photochemical quenching, qP = coefficient of photochemical quenching, NPQ = non-photochemical quenching, Y(II) = PSII quantum yield, D = dissipated thermally, Y(NPQ) = quantum yield of regulated energy dissipation, Y(NO) = quantum yield of non-regulated energy dissipation, Y(I) = photochemical quantum yield of PSI, Y(NA) = non-photochemical quantum yield of PSI, Y(ND) = non-photochemical quantum yield of PSI. *, ** and *** represent significant differences at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively.

3.2 Photosynthesis Rate, Transpiration Rate, Stomatal Conductance and Water Use Efficiency

In the presence of 50 μM Cd, there were decreases of 49.31% in photosynthetic rate, 69.52% in transpiration rate and 59.89% in stomatal conductance on leaves of pea plants as compared to controls (Figs. 2A–2C). At 200 μM Cd concentration, photosynthetic rate decreased by 63.87%, transpiration rates by 86.60% and stomatal conductance by 80.00% as compared to controls. In plants treated with both 50 μM $CdCl_2$ and 50 μM SNP, photosynthesis, transpiration rates and stomatal conductance were partially recovered by 97.75%, 60.89% and 43.00%, respectively, compared to those treated only with 50 μM $CdCl_2$. Plants treated with 200 μM $CdCl_2$ and 50 μM SNP showed 124.66%, 126.62% and 88.12% recovery in photosynthetic rate, transpiration rates and stomatal conductance, respectively, compared to plants treated only with 200 μM Cd (Figs. 2A–2C). Treatment of 50 or 200 μM Cd increased water use efficiency by 5.00% or 125.18%, respectively, as compared to the control treatments. However, plants treated with 50 or 200 μM $CdCl_2$ along with 50 μM SNP showed an enhancement in water use efficiency by 88.89% and 8.34%, respectively, compared to plants treated only with 50 and 200 μM $CdCl_2$ (Fig. 2D). Moreover, MLR also revealed that Cd application resulted in a decline in photosynthetic rate,

transpiration rate, and stomatal conductance of the plants, whereas Cd treatment with SNP significantly increased these parameters on pea plants (Table 1). This is, the relationships between the Cd levels *vs.* the photosynthetic rate, transpiration rate, and stomatal conductance of the plants were negative. However, those between the Cd levels + SNP *vs.* the photosynthetic rate, transpiration rate, and stomatal conductance of the plants were positive (Table 1).

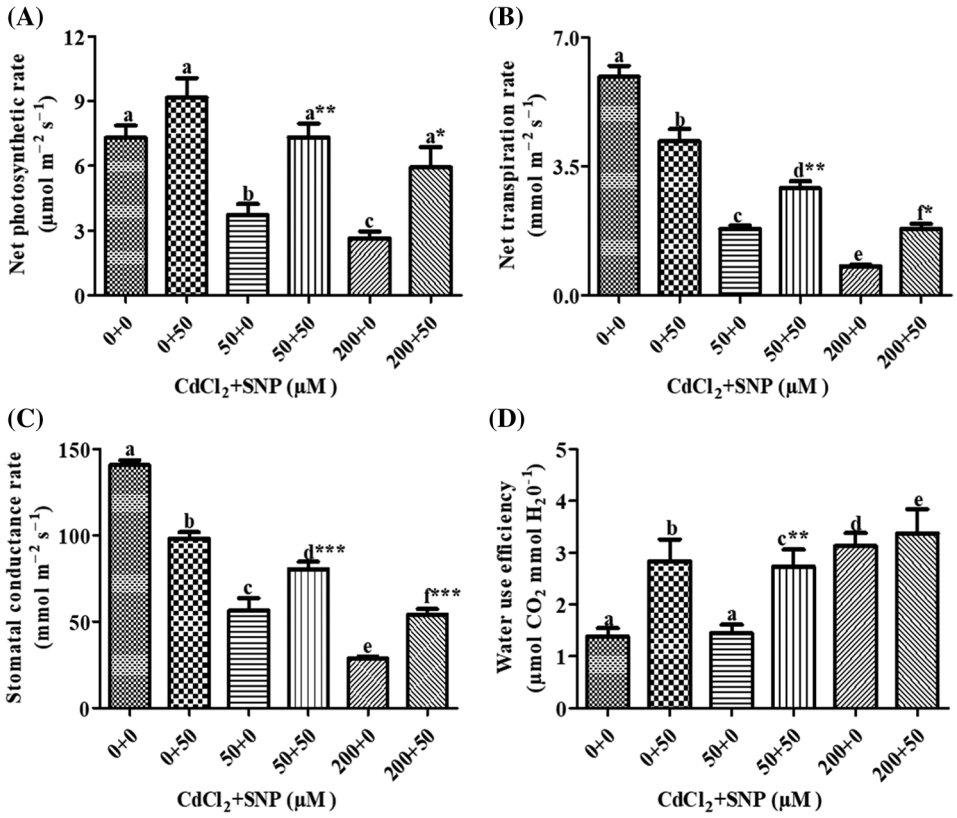

Figure 2: Effect of Cd, SNP and their combinations on photosynthetic rate (A), transpiration rate (B), stomatal conductance rate (C) and water use efficiency (D) In leaves of pea seedlings. Values are means ±1 standard error (SE). Different letters represent significant differences compared to the control treatment at $P \leq 0.05$. *, ** and *** represent significant differences between the Cd + SNP treatment and the Cd treatment at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively

3.3 SNP Maintains Chlorophyll Fluorescence under Cd Stress

Fo, Fm and Fv/Fm were used to measure the photochemical efficiency and PSII activity. Compared to the control, Fo increased by 7.36% or 18.45% in leaves of 50 or 200 μM $CdCl_2$ treated plants, respectively. Addition of 50 μM SNP along with the 50 or 200 μM $CdCl_2$ treatment resulted in a significant decrease of 44.00% or 37.24%, respectively, as compared to plants treated with 50 or 200 μM $CdCl_2$ alone (Fig. 3A). Compared to the control, Fm decreased by 35.71% or 46.47% in the 50 or 200 μM $CdCl_2$ treatment, respectively. Addition of the 50 μM SNP treatment resulted in a significant increase of 27.85% or 30.88% in comparison to plants treated with 50 or 200 μM $CdCl_2$, respectively (Fig. 3B). In contrast, Fv/Fm in 50 or 200 μM Cd-treated pea leaves decreased by 16.56% or 40.53%, respectively, as compared to the control. However, the 50 μM SNP treatment inhibited the decrease of Fv/Fm by 35.79% or 64.48% as compared to plants treated with 50 or 200 μM Cd alone, respectively (Fig. 3C). qN in 50 or 200 μM

$CdCl_2$-treated plants increased by 87.36% or 2.14 folds, respectively, compared to the control. However, 50 µM SNP treatment along with 50 or 200 µM $CdCl_2$ inhibited the increase of qN by 42.76% or 53.00%, respectively (Fig. 3D).

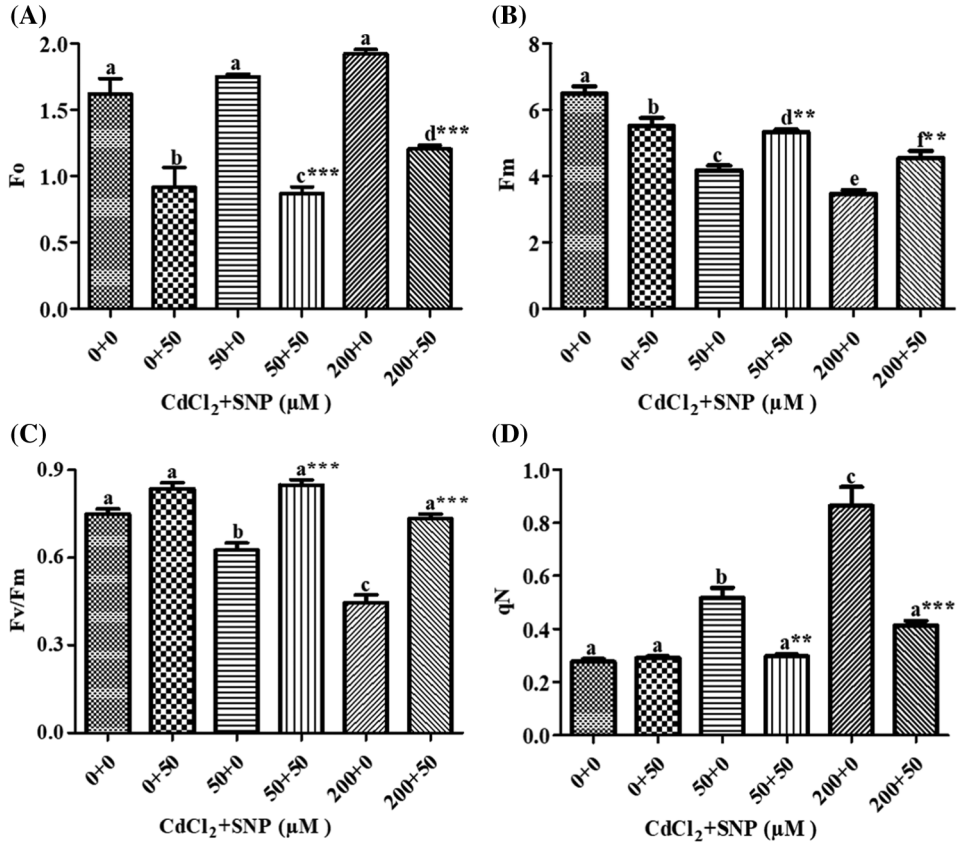

Figure 3: Effect of Cd, SNP and their combinations on Fo (A), Fm (B), Fv/Fm (C), and qN (D) on leaves of pea seedlings. Values are means ±1 standard error (SE). Different letters represent significant differences at $P \leq 0.05$ as compared to the control. *, ** and *** represent significant differences between the Cd + SNP treatment and the Cd treatment at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively

In our study, qL increased by 103.35% and decreased by 42.67% on leaves of 50 and 200 µM $CdCl_2$ treated seedlings, respectively, compared to the control (Fig. 4A). In turn, 50 µM SNP treatment decreased qL by 63.50% and increased it by 1.86 folds in comparison to the 50 and 200 µM $CdCl_2$ treatments, respectively (Fig. 4A). qP decreased by 13.65% and 70.65% on leaves of 50 and 200 µM $CdCl_2$-treated seedlings, respectively, in comparison to the control. The 50 µM SNP treatment decreased qP by 18.15% and 2.48 folds as compared to the 50 and 200 µM $CdCl_2$ treatments, respectively (Fig. 4B). NPQ increased by 82.28% and 137.36% in 50 and 200 µM $CdCl_2$ treatment, respectively, over values on the control. However, 50 µM SNP treatment inhibited the increase of NPQ by 29.45% and 32.02% as compared to 50 and 200 µM $CdCl_2$ treatments, respectively (Fig. 4C). Y(II) was decreased by 34.72% and 86.00% to the 50 and 200 µM $CdCl_2$ treatements, respectively in comparison to the control (Fig. 4D), However, 50 µM SNP treatment inhibited the decreases in Y(II) by 78.63% and 5 folds as compared to 50 and 200 µM $CdCl_2$ treatments, respectively (Fig. 4D).

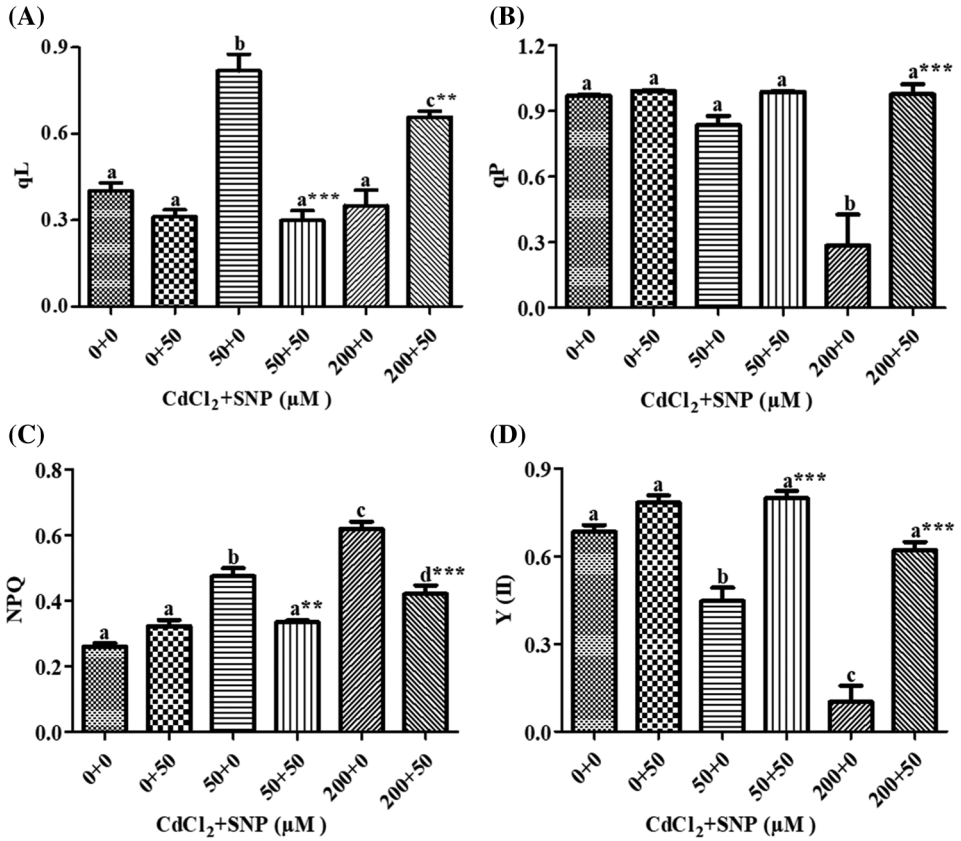

Figure 4: Effect of Cd, SNP and their combinations on qL (A), qP (B), NPQ (C), and Y(II) (D) on leaves of pea seedlings. Values are means ±1 standard error (SE). Different letters represent significant differences at $P \leq 0.05$ as compared to the control. *, ** and *** represent significant differences between the Cd + SNP treatment and the Cd treatment at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively

Our results showed that on leaves of 50 and 200 μM $CdCl_2$-treated seedlings, dissipated thermally D increased by 58.35% and 1.26 folds, respectively, (Fig. 5A); Y(NPQ) increased by 1.38 folds and 4.28 folds, respectively, (Fig. 5B), and Y(NO) increased by 59.16% and 121.69%, respectively, (Fig. 5C), in comparison to controls. However, 50 μM SNP treatment inhibited (1) the increase in D by 59.14% and 50%, (2) the increase in Y(NO) by 61.62% and 51.96%, and (3) the decrease in Y(NPQ) by 68.72% and 67.23% in comparison to the 50 and 200 μM $CdCl_2$ treatments, respectively (Figs. 5A–5C).

Moreover, MLR also revealed that Cd application resulted in a decline of Fm, Fv/Fm, Y(II) and qP, and an increase of Fo, qN, qL, NPQ, D, Y(NPQ) and Y(NO) of the fluorescence parameter. In turn, the Cd treatment with SNP significantly increased Fm, Fv/Fm, qP, and Y(II), and decreased Fo, qN, qL, NPQ, D, Y(NPQ) and Y(NO) of the fluorescence parameter on pea plants. The relationship between the Cd levels *vs.* (1) Fm, Fv/Fm, Y(II) and qP was negative, and (2) Fo, qN, qL, NPQ, D, Y(NPQ) and Y(NO) of the fluorescence parameter was positive (Table 1). In turn, the relationship between the SNP treatment *vs.* (1) Fm, Fv/Fm, qP, and Y(II) was positive, and (2) Fo, qN, qL, NPQ, D, Y(NPQ) and Y(NO) of the fluorescence parameter was negative (Table 1).

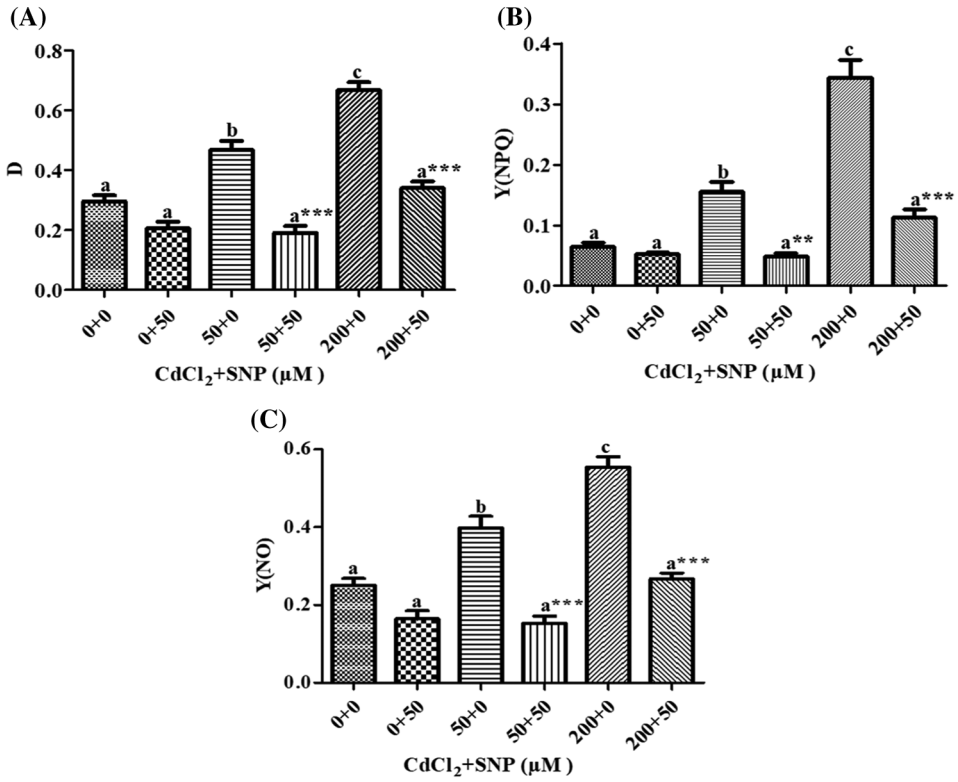

Figure 5: Effect of Cd, SNP and their combinations on D (A), Y(NPQ) (B) and Y(NO) (C) on leaves of pea seedlings. Values are means ±1 standard error (SE). Different letters represent significant differences at $P \leq 0.05$ as compared to the control. *, ** and *** represent significant differences between the Cd + SNP treatment and the Cd treatment at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively

3.4 Effect of SNP on PSI under Cd Stress

In our study, Y (I) decreased by 30.96% and 62.80% on leaves of 50 and 200 µM $CdCl_2$-treated plants, respectively, as compared to control (Fig. 6A). Addition of 50 µM SNP along with Cd did not show a significant change in Y (I) when compared to the Cd treatments. Furthermore, Y(NA) decreased by 45.89% and 23.63% due to the 50 and 200 µM $CdCl_2$ treatments, respectively. Addition of 50 µM SNP to50 and 200 µM $CdCl_2$ further reduced Y(NA) by 60.23% and 22.77% compared to the 50 and 200 µM $CdCl_2$ treatment, respectively (Fig. 6B). Furthermore, Y(ND) increased by 46.63% and 88.76% due to the 50 and 200 µM $CdCl_2$ treatments, respectively. Supplementation of 50 µM SNP with 50 and 200 µM $CdCl_2$ did not cause any significant change in Y(ND) in comparison to the 50 and 200 µM $CdCl_2$ treatments (Fig. 6C).

MLR revealed that Cd treatment caused reduction in Y(I) and Y(NA) but increased Y(ND). However, the application of the nitric oxide donor (SNP) along with Cd significantly increased Y(I) and Y(ND) and decreased Y(NA). The relationships between the Cd levels *vs.* Y(I) and Y(NA) were negative while that *vs.* Y(ND) was positive (Table 1). In turn, the relationship between SNP *vs.* Y(I) and Y(ND) was positive while that *vs.* Y (NA) of PSI was negative (Table 1).

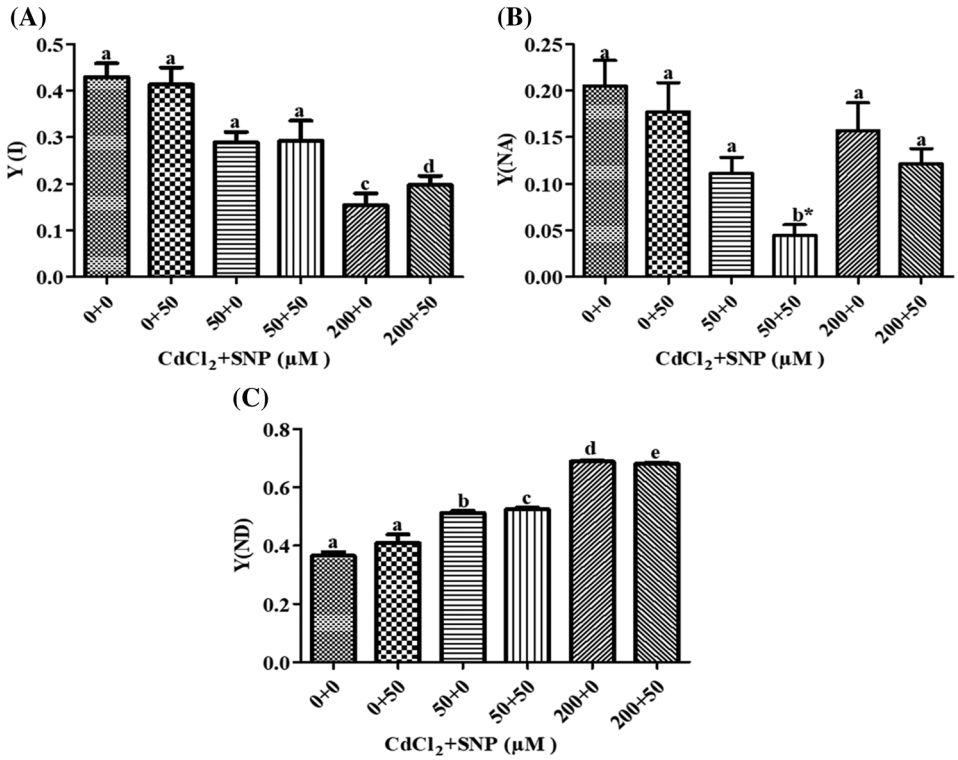

Figure 6: Effect of Cd, SNP and their combinations on PSI Y(I) (A), Y(NA) (B), and Y(ND) (C) in leaves of pea seedlings. Values are means ±1 standard error (SE). Different letters represent significant differences at $P \leq 0.05$ as compared to the control. *, ** and *** represent significant differences between the Cd + SNP treatment and the Cd treatment at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively

4 Discussion

Photosynthesis is considered as one of the most important physiological processes in plants. The whole metabolism of plants specifically or by implication depends on this process and hence any change in photosynthetic rate will automatically affect the remaining plant processes. Cd can inhibit photosynthesis and induce damage and dysfunction of chloroplast. This decrease may be because of (i) inhibition of photosynthetic electron transport chain [31], (ii) inhibition of the enzymes required for Chl biosynthesis [31], (iii) disturbance in the PSII reaction center [32], (iv) replacement of central Mg^{2+} of Chl [33] and (v) reduction in P uptake which is involved in pigment biosynthesis [33]. The Cd could harm chloroplast submicroscopic structure, through damaging grana stacking structure [34,35]. The ability of the chloroplast for capturing light energy is greatly decrease in presence of Cd, thus affecting the role of many functions associated with photosynthesis [36].

Our results showed less concentration of photosynthetic pigments in leaves of Cd-treated *Pisum sativum*. This could be because of Cd-led increases in the activity of chlorophyllase, inhibition of the enzymes involved in the biosynthesis of photosynthetic pigments and destruction of photosynthetic pigments by oxidative stress [37]. In the present study, a significant loss in chlorophyll concentration was seen in Cd-treated plants. Chl b was affected more strongly than Chl a. Chlorophyll concentration was also strongly diminished in *Oryza sativa* [38], *Hordeum vulgare* [39], *Lycopersicon esculentum* [40–42], *Zea mays* [43] and *Brassica oleracea* [44,45] exposed to Cd. A putative debasement of chlorophyll and additionally the hindrance of its biosynthesis were proposed to be responsible for the inhibition of photosynthesis and growth due to Cd [36]. In the present study, NO supplementation to Cd stressed

plants reverted chlorophyll loss in pea which can be corroborated with findings in maize [46], lettuce [47,48], fava bean [31] and mustard [49] under Cd toxicity. Increased Chl due to NO supplementation could be due to reduced oxidative stress and damage to chlorophyll pigments in Cd-treated plants (Fig. 1A). NO inhibits photosynthetic pigment degradation by protecting the photosynthetic membrane, Rubisco, cytochrome b6/f, and D1 and D2 proteins [50]. Carotenoids by acting as light-harvesting pigments can protect chlorophyll and membranes from damage by quenching triplet chlorophyll and removing oxygen from the excited chlorophyll [51]. Carotenoids enhancement in pea plants exposed to 50 µM SNP with 50 µM Cd and 200 µM Cd-treated plants (Fig. 1B) may reflect an attempt to protect chlorophyll and the photosynthetic mechanical assembly from the photo oxidative destruction of Cd toxicity [52]. Our results suggested Cd-induced inhibition of the photosynthesis rate, transpiration rate, and stomatal conductance in pea plants (Figs. 2A–2C). Similar to our results, several studies have shown Cd-led inhibition in photosynthesis rate, transpiration rate, and stomatal conductance in pea plants [53]. The observed amelioration in photosynthesis rate, transpiration rate, stomatal conductance and water use efficiency in presence of SNP could be due to the impact of NO on stomatal closure (Figs. 2A–2C).

PSII is one of the prime targets of Cd toxicity. Chl fluorescence was studied to evaluate the effects of Cd, SNP and the combination of Cd and SNP on PSII on Cd-stressed pea plants. Similar to prior studies, Cd diminished PSII values and decreased electron yield of PSII [9,54]. The F_v/F_m, representing the maximal efficiency of excitation energy capture by the "open" PSII reaction center, is usually used as a stress indicator on plants [55]. In this study, the Fv/Fm ratio decreased with Cd treatment (Fig. 3C). In pea plants exposed to 50 and 200 µM Cd, the increase of Fo and the decrease of Fm resulted in a reduction of the Fv/Fm ratio (Fig. 3A–3C). An increase of Fo points to photo damage, and a decline in Fm, reflect an enhanced non-radiative energy. The Fv/Fm was reduced in several plant species, including pea, exposed to Cd [56]. The decrease in F_v/F_m and PSII efficiency suggested that Cd stress led to the inhibition of the PSII photoactivation. This might have been due to damage of the antennae pigments and the limitation of Q_A (quinone) reoxidation due to the decrease or partial blockage of the electron transport from PSII to PSI [57]. The exogenous application of SNP increased the F_v/F_m ratio, which specifies that the plant is healthy, and not suffering from photoinhibition (Fig. 3C).

Under various stress conditions, an increment in NPQ and qN can be associated with photoinactivation of PSII reaction centers, that leads to an oxidative damage to the reaction centers and an increase in Fo [58]. In the present study qN and NPQ increments were with increases in F_v/F_m under Cd toxicity (Figs. 3D and 4C). Use of SNP along with Cd led to a decrease in qN and NPQ through regulating photochemistry. It can be considered as an additional mechanism to adjust for the excess of absorbed light energy; therefore, SNP counters photoinhibition of PSII caused by Cd toxicity. Under Cd stress, SNP increased Fv/Fm and photochemical efficiency, and decreased NPQ and qN of PSII in ryegrass seedling leaves treated with $NaHCO_3$ [59]. SNP restored the chlorophyll fluorescence. This might have been due to its role in (i) protecting the pigment systems from oxidative damage, (ii) keeping up the chlorophyll biosynthesis.

PSI is considered to be less sensitive to heavy metals; however, apart from a few exceptions [35], earlier studies generally preferred several artificial electron donors and inhibitors to study the PSI activity [16,60] under heavy-metal stress, which may itself affect the PSI activity. Therefore, in the current study we used P700 absorbance measurements, which made direct, noninvasive monitoring of the PSI photochemistry in whole leaves without using electron donors or inhibitors. Our results clearly indicated that Cd decreased PSI photochemistry. Significant damages were observed to the photosynthetic electron transport at concentrations of 50 and 200 µM $CdCl_2$ (Fig. 6A). PSI efficiency measurements on the Cd-treated plants revealed decreased yields of PSI Y(I), and Y(NA), and increased Y(ND), which can be caused by an increased cyclic electron flow (CEF) around the PSI (Figs. 6A–6C). Earlier reports demonstrated that Cd caused iron deficiency in cell organelles which is probably a reason for PSI damage [61,62]. Long-term iron deficiency resulted in ROS production in thylakoids which primarily damage iron-sulphur centres

(PSI) and LHC I antennae [63]. Cd-induced damage to PSI has been noticed in *Cucumis sativus* [64,65] and *Triticum aestivum* [66]. Unlike the chlorophyll concentration and PSII, SNP treatment did not show any ameliorative effects on PSI parameters. These results suggest that Cd stress caused damage to both PSI and PSII in *Pisum sativum*. They also suggest that exogenous NO application is useful in mitigating the Cd-induced damage to photosynthesis on pea seedlings because of its ameliorating effects on the photosynthetic pigment concentrations and PSII.

Funding Statement: The authors thank to DBT for the financial support of BUILDER Project No. BT/PR-9028/INF/22/193/2013. EAK acknowledge the receipt of UGC-MANF for minority students, MM acknowledge the award of UGC-PDF for women, and PS thanks to UGC-FRP during the period of this work.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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