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Nitric oxide pretreatment enhances antioxidant defense and glyoxalase systems to confer PEG-induced oxidative stress in rapeseed

Mirza Hasanuzzaman[a], Kamrun Nahar[b], Md. Shahadat Hossain[c], Taufika Islam Anee[a], Khursheda Parvin[d] and Masayuki Fujita[c]

[a]Department of Agronomy, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh; [b]Department of Agricultural Botany, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh; [c]Laboratory of Plant Stress Responses, Faculty of Agriculture, Kagawa University, Kagawa, Japan; [d]Department of Horticulture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh

ABSTRACT

Nitric oxide (NO) is dynamic molecule implicated in diverse biological functions demonstrating its protective effect against damages provoked by abiotic stresses. The present study investigated that exogenous NO pretreatment (500 µM sodium nitroprusside, 24 h) prevented the adverse effect of drought stress [induced by 10% and 20% polyethylene glycol (PEG), 48 h] on rapeseed seedlings. Drought stress resulted in reduced relative water content with increased proline (Pro) level. Drought stress insisted high H_2O_2 generation and consequently increased membrane lipid peroxidation which are clear indications of oxidative damage. Drought stress disrupted the glyoxalase system too. Exogenous NO successfully alleviated oxidative damage effects on rapeseed seedlings through improving the levels of nonenzymatic antioxidant pool and upregulating antioxidant enzymes' activities. Improvement of glyoxalase system (glyoxalase I and glyoxalase II activities) by exogenous NO was significant to improve plants' tolerance. Nonetheless, regulation of Pro level and improvement of plant–water status were vital to confer drought stress tolerance.

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KEYWORDS
Osmotic stress; plant–water relations; reactive oxygen species; signaling molecule; water stress

Introduction

In the present world, crops are suffering from drought stress, and its duration and severity are also increasing day by day due to climatic changes and thus imposing a continuous threat to food production. Drought stress hampers plant productivity through affecting normal plant growth and physiology, different biochemical processes, and yields (Raza et al. 2016; Hasanuzzaman et al. 2016; Cao et al. 2017). Plants face oxidative stress under water deficit conditions by producing an excess amount of reactive oxygen species (ROS) which causes damage to biological molecules and cellular organelles. These damages ultimately result in cell death (Hasanuzzaman et al. 2013; Nahar et al. 2015a, 2015b). Plants naturally have an antioxidant defense system for scavenging excess ROS, and this can protect the plant from oxidative damage (Nahar et al. 2015b; Kim et al. 2017). In this system, both 2013; Nahar et al. 2015a; Kim et al. 2017; Wu et al. 2017). Methylglyoxal (MG) is produced in eukaryotic cells as an intermediate product in glycolysis pathway; its excess production is toxic and it inhibits cell proliferation and causes protein and lipid degradation (Yadav et al. 2005; Nahar et al. 2015b; Sankaranarayanan et al. 2017). Based on some recent studies, MG was found to act as signaling molecules which may act as important biomarkers for plant stress responses (Kaur et al. 2014, 2017; Li 2016; Sankaranarayanan et al. 2017). However, excess production of MG has been noticed in plants that are under drought stress (Alam et al. 2013; Nahar et al. 2015b; Sankaranarayanan et al. 2017). Plants also can detoxify this excess MG through the activity of glyoxalase I (Gly I) and glyoxalase II (Gly II) in glyoxalase system (Nahar et al. 2015b; Hasanuzzaman et al. 2017a, 2017b; Sankaranarayanan et al. 2017).

Nitric oxide (NO) is an endogenous signaling molecule in plants regulating different biological functions because of its high diffusible property (Qiao et al. 2014). NO plays its signaling role in stress conditions by regulating different physiological activities such as germination, mitochondrial function, floral regulation, photosynthesis, proline (Pro) accumulation, stomatal movement, etc. (Boogar et al. 2014; Domingos et al. 2015; Hasanuzzaman et al. 2016; Melo et al. 2016). NO may act as an antioxidant or a source of reactive nitrogen species having greater oxidizing potential which also takes part in many physiological processes (Vandelle and Delledonne 2011; Domingos et al. 2015; del Rio 2015; Hasanuzzaman et al. 2016). al. 2013; Hasanuzzaman et al. 2016; Sahay and Gupta 2017). It is also well documented that NO can reduce H_2O_2 and lipid peroxidation under drought stress (Zhang et al. 2016). NO mitigates oxidative damage and acts as an antioxidant (Zimmer-Prados et al. 2014; Hasanuzzaman et al. 2016; Zhang et al. 2016; Sahay and Gupta 2017). Roles of NO have been demonstrated in reducing ROS-induced cytotoxic activities such as inhibition of cell death, ion leakage, and DNA fragmentation (Zhang et al. 2016) which was also noticed in *Solanum tuberosum* (Hayat et al. 2011). NO improves relative water content (RWC), increases activities of superoxide dismutase (SOD) and ascorbate peroxidase (APX), and reduces ion leakage significantly in the plant under drought stress (Hatamzadeh et al. 2015; Zhang et al. 2016). Moreover, NO also enhances the activities of antioxidant enzymes and results in a decreased lipid peroxidation under drought stress (Astier and Lindermayr 2012; Fan et al. 2012; Zhang et al. 2012; Kovacs and Lindermayr 2013).

CONTACT Mirza Hasanuzzaman mhzsauag@yahoo.com

Although several research works have been carried out on the effect of NO on plant drought tolerance, the actual mechanisms are yet to be elucidated. Considering these facts, the present study was undertaken to investigate the role of supplemental NO in drought tolerance. We have shown how antioxidant defense and glyoxalase system are regulated by NO under drought stress.

Materials and methods

Test plant and applied treatments

Clean and uniform seeds of rapeseed (*Brassica napus* cv. BINA Sarisha 3) were sown in Petri plates containing filter paper. Petri plates were kept in a growth chamber with the following conditions: μmol photon m^{-2} s^{-1}, 25 ± 2°C, and 65–70% relative humidity. Hyponex solution (Hyponex, Japan) (diluted by 10,000 times) was used as a nutrient solution. After 11 days of sowing, a set of seedlings were pretreated with 500 μM sodium nitroprusside (SNP) contained h. A set of preliminary experiments were done to determine the appropriate dose of SNP. Among 125, 250, 500, 750, and 1000 μM, we found that 500 μM SNP could provide better protection without any toxic effect to pants. The doses below that concentration could not show any protection. SNP-pretreated and non-pretreated plants were subjected to drought stress applying 10% and 20% polyethylene glycol (PEG-6000) for 48 h. Control plants were provided with Hyponex solution. Maintaining the identical growing environment, the experiment was repeated three times.

Ascorbate and GSH assay

Ethylenediaminetetraacetic acid (EDTA) (1 mM) containing metaphosphoric acid solution (5%) was used to extract harvested fresh rapeseed leaves. After centrifugation (11,500×*g*), the same supernatant was used for both ascorbate (AsA) and glutathione (GSH) assays. In spectrophotometer, the wavelength of 265 nm was selected to determine the content of AsA, where different reacting solutions were used as mentioned by Huang et al. (2005). After neutralizing with 0.5 M potassium phosphate (K–P) buffer (pH 7.0), 0.5 units of ascorbate oxidase (AO) in 100 mM K–P buffer (pH 7.0) was added and read using a spectrophotometer. Using standard curve, AsA content was calculated. Neutralizing supernatant with 0.5 M K–P buffer (pH 7.0), the contents of total GSH and glutathione disulfide (GSSG) were determined at 412 nm (Yu et al. 2003; Paradiso et al. 2008). For measuring total GSH, the assay buffer contained 5,5′-dithio-bis 2-nitrobenzoic acid (DTNB), NADPH, and GR. For GSSG measurement, reaction of 2-vinylpyridine was added to remove the GSH. Standard curve with their (GSH and GSSG) known concentrations were made to facilitate the final calculation. GSH content was the result of total GSH content minus the GSSG content.

Enzyme extraction

One milliliter of extraction buffer formulated with K–P buffer, KCl, AsA, *β*-mercaptoethanol, and glycerol was used to homogenize 0.5 g of rapeseed leaves (fresh). For not more than 10 min, this homogenate underwent centrifugation at a speed of 11,500×g. The same supernatant was used for protein estimation (Bradford 1976) as well as crude solution for the enzyme activity assay.

Enzyme assay

Ascorbate peroxidase (APX; EC: 1.11.1.11) activity: The APX activity was measured mixing the enzyme solution with 50 mM K–P buffer (pH 7.0), 500 μM AsA, 0.1 mM H_2O_2, and 0.1 mM EDTA. This mixture was read at 290 nm to measure the alteration of absorbance (Nakano and Asada 1981).

Monodehydroascorbate reductase (MDHAR; EC: 1.6.5.4) activity: The MDHAR enzyme assay mixture was prepared by adding 50 mM Tris–HCl buffer (pH 7.5), 0.2 mM NADPH, 2.5 mM AsA, and 0.5 units of AO (Hossain et al. 2010). A decrease in absorbance at 340 nm was observed for 60 s.

Dehydroascorbate reductase (DHAR; EC: 1.8.5.1) activity: The mixture of 50 mM K–P buffer (pH 7.0), 2.5 mM GSH, and 0.1 mM DHA with enzyme solution was read at 265 nm to observe the change in absorbance (Nakano and Asada 1981).

Glutathione reductase (GR; EC: 1.6.4.2) activity: The GR enzyme assay mixture was prepared by adding 0.1 M K–P buffer (pH 7.0), 1 mM EDTA, 1 mM GSSG, and 0.2 mM NADPH and then change in absorbance was recorded at 340 nm (Hasanuzzaman et al. 2011).

Glutathione peroxidase (GPX; EC: 1.11.1.9) activity: The GPX enzyme assay mixture contained 100 mM K–P buffer (pH 7.0), 1 mM EDTA, 1 mM NaN_3, 0.12 mM NADPH, 2 mM GSH, 1 unit GR, and 0.6 mM H_2O_2 (Elia et al. 2003; Hasanuzzaman and Fujita 2013). We recorded the change in absorbance at 340 nm.

Glutathione *S*-transferase (GST; EC: 2.5.1.18) activity: The GST enzyme assay mixture was prepared by adding 100 mM Tris–HCl buffer (pH 6.5), 1.5 mM GSH, and 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) (Hasanuzzaman and Fujita 2013). At 340 nm, the change in absorbance was monitored.

Catalase (CAT; EC: 1.11.1.6) activity: The CAT enzyme assay mixture was prepared by adding 50 mM K–P buffer (pH 7.0) and 15 mM H_2O_2. The absorbance was recorded in 240 nm (Hasanuzzaman et al. 2011).

Glyoxalase I (Gly I; EC: 4.4.1.5) activity: The Gly I enzyme assay mixture contained 100 mM K–P buffer (pH 7.0), 15 mM magnesium sulfate, 1.7 mM GSH, and 3.5 mM MG (Hasanuzzaman et al. 2014) which was read at 240 nm.

Glyoxalase II (Gly II; EC: 3.1.2.6) activity: The Gly II enzyme assay mixture was prepared by adding 100 mM Tris–HCl buffer (pH 7.2), 0.2 mM DTNB, and 1 mM S-D-lactoylglutathione (SLG) (Hasanuzzamanlactoylglutathione (SLG) (Hasanuzzaman et al. 2014). This mixture was read at 240 nm.

Assaying H_2O_2 content

The procedure mentioned by Yu et al. (2003) was followed to determine H_2O_2 levels. Potassium–phosphate (K–P) buffer of 50 mM concentration and pH 6.5 was selected to homogenize the harvested fresh rapeseed leaves. After centrifugation (at 11,500×*g*), the supernatant had been added to the reaction mixture (0.1% $TiCl_4$ in 20% H_2SO_4). Before centrifuging (11,500×*g*) again for 15 min, the solution was allowed to set for 10 min at normal temperature. In the spectrophotometer,

the wavelength of 410 nm was selected to read the absorbance of the solution.

Estimation of MDA content

The outline explained by Heath and Packer (1968) was followed to estimate malondialdehyde (MDA) content. Trichloroacetic acid (TCA, 5%) was used to homogenize the harvested fresh rapeseed leaves. After homogenization on ice, the homogenate underwent centrifugation (at 11,500×*g*). The extract was then added to the reaction mixture (0.5% thiobarbituric acid (TBA) dissolved in 20% TCA) followed by boiling (95°C) for 30 min. The hot solution was then kept on ice for quick cooling, and after cooling, it was exposed to centrifugation again for 15 min at the same speed mentioned previously. Absorbance was measured at 532 and 600 nm wavelengths. The second absorbance was measured to have actual (nonspecific turbidity-free) absorbance, by deducting it from the first one. The unit used to express the results was nmol g^{-1} fresh weight.

Determination of RWC

The method described by Barrs and Weatherly (1962) was followed for estimating the leaf RWC. Immediately after weighing (fresh weight, FW), leaves were left to float in distilled water and for 8 h. Then, again they were weighed (turgid weight, TW) and kept in a drier at 80°C. After 48 h, dry leaves were weighed to obtain the dry weight (DW). The following formula was used to do the calculation:

$$\mathrm{RWC}\,(\%) = \left[\frac{(\mathrm{FW}-\mathrm{DW})}{(\mathrm{TW}-\mathrm{DW})}\right] \times 100.$$

Determination of proline content

Bates et al. (1973) described method of measuring Pro content was followed in our experiment. Harvested fresh leaves had been extracted by 3% sulfosalicylic acid followed by centrifugation (11,500×*g*) for 15 min. The filtrate (2 ml) was added in the reaction mixture (2 ml acid ninhydrin + 2 ml glacial acetic acid). This mixture was placed at water bath at 100°C (1 h). After cooling, 4 ml toluene was added and combined using vortex. Chromophore of toluene was read at 520 nm.

Statistical analysis

The data were obtained using one-way analysis of variance. We tested and then compared mean differences by Fisher's least significant difference (LSD) from three replications. The software XLSTAT v. 2015 was used to perform analysis (Addinsoft 2016). Differences at $P \leq .05$ were regarded as significant.

Results

RWC and Pro content

Leaf RWC significantly declined due to drought exposure (Figure 1(a)) which in turn enhanced the Pro content of drought-stressed seedlings drastically (Figure 1(b)) compared to non-stressed control plants. However, NO pretreatment has been recorded to decrease the Pro content by 30% and 33%, and increase the leaf RWC by 12% and 26% in seedlings facing mild and severe levels of stress, respectively compared to the non-treated drought-stressed seedlings (Figure 1).

Hydrogen peroxide and MDA contents

Both the levels of H_2O_2 and MDA were significantly increased in rapeseed leaves due to exposure to drought stress (Figure 2) and particularly, a noteworthy higher content was recorded at 20% PEG, compared to the control plants. Drought-stressed seedlings which were supplemented with NO donor resulted in 20% and 21% reduction in H_2O_2 content and 21% and 32% reduction in MDA content by 10% and 20% PEG, respectively, as compared to the drought-exposed seedlings without NO donor.

AsA and GSH contents

AsA content was unaffected under severe stress, whereas it was slightly higher (21%) under mild drought stress compared to the control plants (Figure 3(a)). Supplementation with NO increased the contents of AsA significantly compared to seedlings treated with PEG only (drought-stressed). Due to drought exposure, a remarkable enhancement of GSH content was recorded (55% and 46% at 10% and 20% PEG, respectively) as compared with the untreated control plants (Figure 3(b)). However, at both cases, NO-supplemented seedlings showed a significant increase (17% and 12% at 10% and 20% PEG, respectively) in GSH content compared to seedlings subjected to drought stress alone. GSSG content showed dramatic increase under drought stress, while it became lower when the seedlings were supplemented with NO (Figure 3(c)). The ratio of GSH/GSSG declined in the seedlings exposed to drought. However, a higher GSH/GSSG ratio was maintained by the addition of NO donor even in the seedlings under stressed condition (Figure 3(d)).

AsA-GSH pool enzyme activities

Under drought stress, APX activities did not show any change, while the activity increased significantly when the seedlings were supplemented with NO donor (Figure 4(a)). The activity of MDHAR enzyme was higher than that in control plants at mild stress, while it was less under severe stress (Figure 4(b)). DHAR activity increased significantly at both levels of drought stress (Figure 4(c)). When compared with seedlings under drought treatment alone, the activities of both the enzymes (MDHAR and DHAR) enhanced in NO-supplemented seedlings (Figure 4(b,c)). The activity of GR was higher (26% and 23% higher at 10% and 20% PEG exposure, respectively) under drought stress treatment compared to control (Figure 4(d)). Importantly, at both the cases, NO supplementation resulted in higher activities of GR compared to the rapeseed seedlings exposed to drought alone (Figure 4(d)).

Other antioxidant enzyme activities

In response to drought stress, GST activity was shown to be increased. When compared with control treatment, the increase in GST activity was measured as 25% and 31% higher at 10% and 20% PEG, respectively (Figure 5(a)). Exogenous

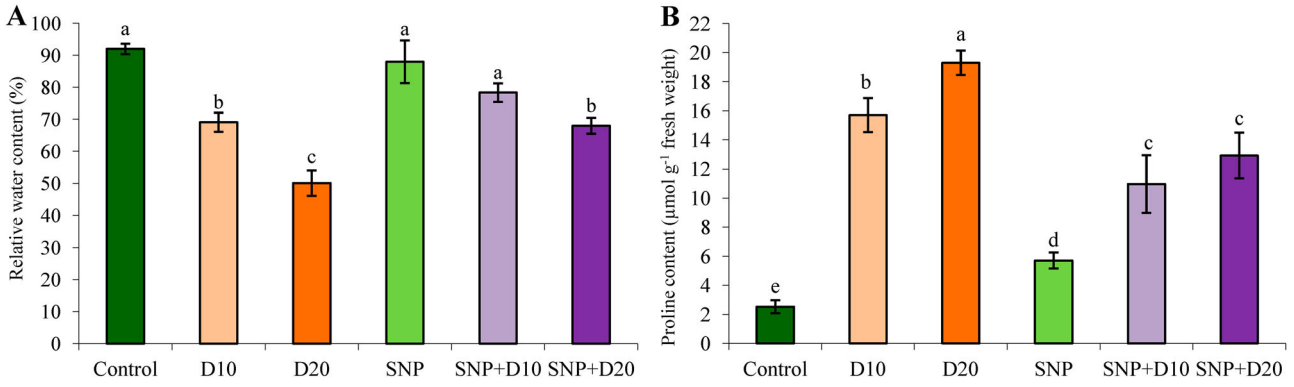

Figure 1. NO donor, SNP-induced changes in RWC (A) and Pro content (B) under drought stress. Treatments: Control, seedlings treated with nutrient solution only; D10, seedlings treated with 10% PEG; D20, seedlings treated with 20% PEG; SNP, seedling treated with 500 μM SNP for 24 h; SNP + D10, 500 μM SNP-pretreated seedlings exposed to 10% PEG; SNP + D20, 500 μM SNP-pretreated seedlings exposed to 20% PEG. Mean (±SD) was calculated from three replicates for each treatment. Vertical bars with different letters are significantly different at $P \leq .05$, determined by Fisher's LSD test.

NO resulted in higher GST activity compared to the stress treatment without NO donor. GPX activity slightly increased (19%) both at mild and severe drought stress (Figure 5(b)). On the other hand, NO supplementation caused a clear increase in the activity even under drought stress. CAT activity markedly decreased at drought stress in a dose-dependent manner (Figure 5(c)). However, upon NO supplementation, the CAT activities returned to the control level.

Glyoxalase system enzyme activities

A slight increase (20% and 15% higher at 10% and 20% PEG, respectively) in the activity of Gly I was observed in seedlings exposed to drought stress (Figure 6(a)). In contrary, upon exposure to drought stress, Gly II activity was shown to be decreased (11% and 30% lower at 10% and 20% PEG, respectively). However, for both the cases, NO application resulted in higher Gly I and Gly II activities compared to the seedlings exposed to drought stress alone (Figure 6).

Discussion

In recent years, NO has been documented as a signaling molecule playing a role in diverse physiological processes in plants involving seed germination, flowering, fruit maturity, organ senescence, and respiratory metabolism (Hasanuzzaman et al. 2016). It is also involved in different physiological and biochemical responses to environmental stresses because of its antioxidant properties (Gupta et al. 2011; Hasanuzzaman et al. 2016). Exogenous application of NO is known to enhance tolerance against abiotic stress including heavy metal toxicity, salinity, and drought (Hasanuzzaman et al. 2011; Hasanuzzaman and Fujita 2013; Oz et al. 2015). However, how NO enhances drought tolerance in plants needs further clarification. In this study, we provided an overview of antioxidant defense and glyoxalase system under drought stress after application of NO donor. Drought stress-induced growth reduction, decrease in water content, nutrient imbalance, and oxidative stress in plants are common metabolic and physiological changes (Hasanuzzaman et al. 2013; Raza et al. 2013). Drought stress primarily affects the plant–water relations and to cope with water shortage condition, the plant synthesizes osmolytes (Pro, glycinebetaine, and sugars) (Hasanuzzaman et al. 2013; Ahmad et al. 2014). In this study, RWC decreased in a dose-dependent manner in rapeseed seedlings under drought stress. Consequently, Pro content increased in the same way to adjust the water balance inside the cell (Figure 1(a)). Our results are in agreement with Nahar et al. (2015b) who reported a drought-induced reduction in RWC and increase in Pro content in mung beans under water deficit conditions. However, application of SNP increased RWC and thus reduced Pro content under drought stress, implying that NO could improve water status of plants under drought stress by maintaining

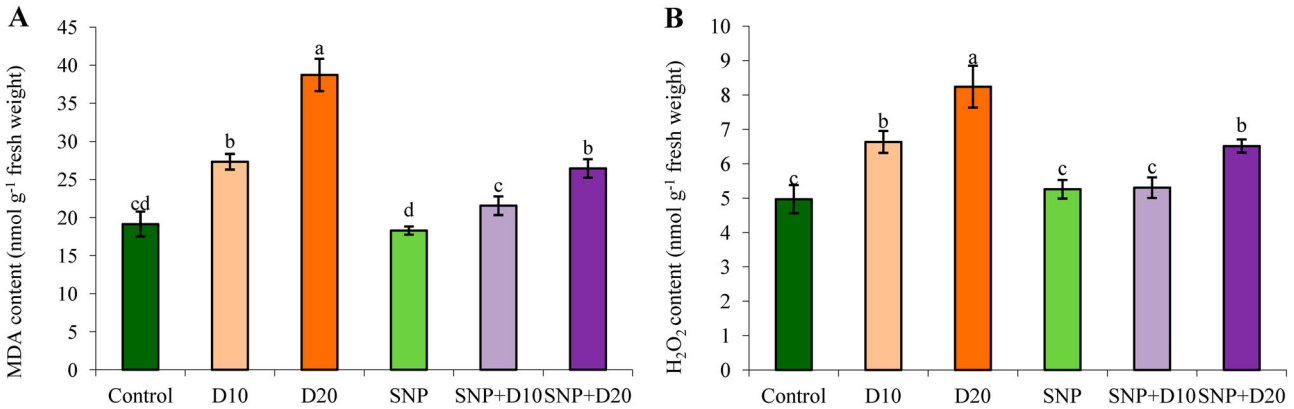

Figure 2. NO donor, SNP-induced changes in MDA content (A) and H_2O_2 content (B) under drought stress. Treatments: Control, seedlings treated with nutrient solution only; D10, seedlings treated with 10% PEG; D20, seedlings treated with 20% PEG; SNP, seedlings treated with 500 μM SNP for 24 h; SNP + D10, 500 μM SNP-pretreated seedlings exposed to 10% PEG; SNP + D20, 500 μM SNP-pretreated seedlings exposed to 20% PEG. Mean (±SD) was calculated from three replicates for each treatment. Vertical bars with different letters are significantly different at $P \leq .05$, determined by Fisher's LSD test.

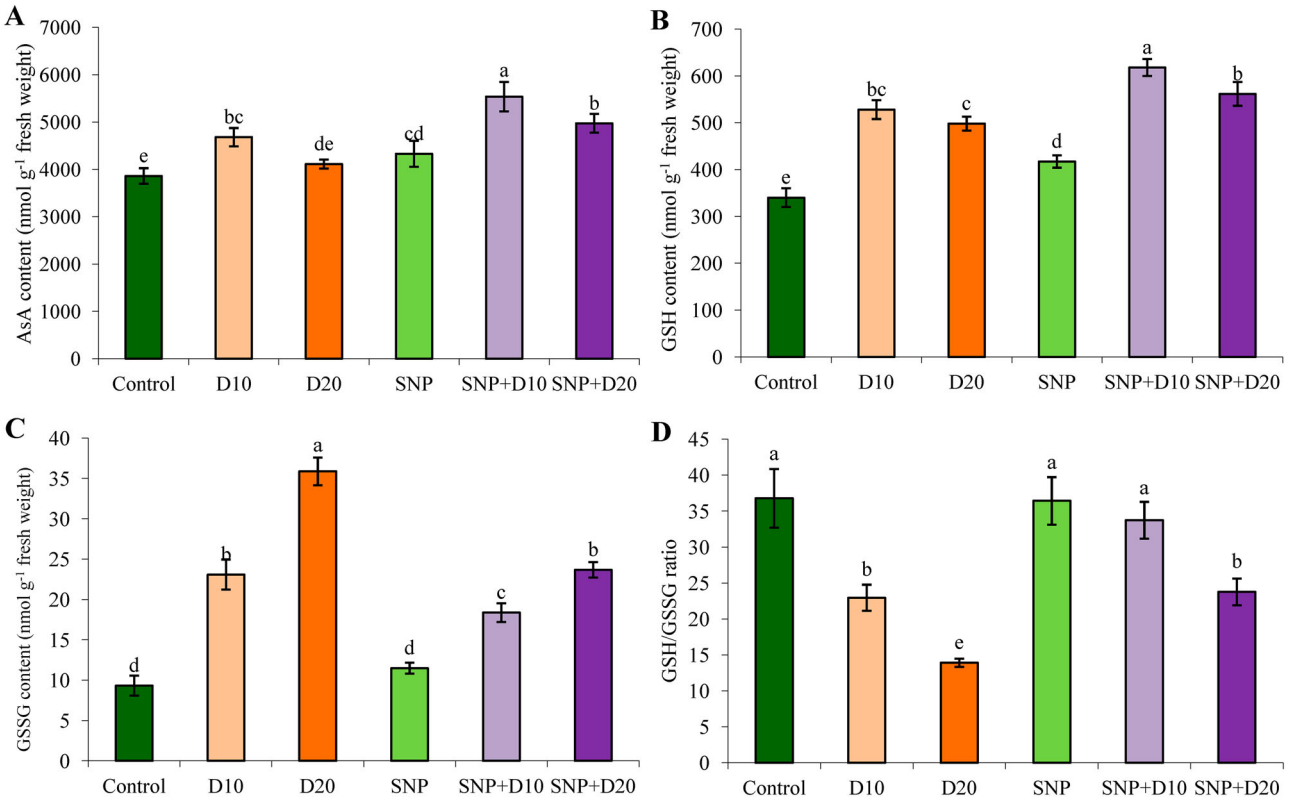

Figure 3. NO donor, SNP-induced changes in AsA (reduced ascorbate) content (A), GSH (reduced glutathione) content (B), GSSG (oxidized glutathione) content (C), and GSH/GSSG ratio (D) under drought stress. Treatments: Control, seedlings treated with nutrient solution only; D10, seedlings treated with 10% PEG; D20, seedlings treated with 20% PEG; SNP, seedling treated with 500 µM SNP for 24 h; SNP + D10, 500 µM SNP-pretreated seedlings exposed to 10% PEG; SNP + D20, 500 µM SNP-pretreated seedlings exposed to 20% PEG. Mean (±SD) was calculated from three replicates for each treatment. Vertical bars with different letters are significantly different at $P \leq .05$, determined by Fisher's LSD test.

osmolytes synthesis (Figure 1(a)). Ke et al. (2013) reported that application of NO lowered the cell solutes and increased the water potential, and thus improved osmoregulation in tobacco callus under osmotic stress. Furthermore, NO was able to enhance salt stress tolerance in Chinese cabbage by reducing Pro content under stress condition. The reduction of Pro content was due to increased activity of Pro dehydrogenase induced by NO (López-Carrión et al. 2008).

Drought creates oxidative stress mainly by interrupting electron flow during photosynthesis (Cruz de Carvalho 2008), and level of oxidative damages is often measured by MDA (indicator of lipid peroxidation) content and ROS level including H_2O_2. In this experiment, higher MDA and H_2O_2 content were found compared to control in drought-stressed seedlings, which means higher oxidative damage in plants (Figure 2). In addition to that, with the increase in drought level using higher PEG amount, level of oxidative damage was also increased (Figure 2). Drought-induced oxidative damage (indicated by higher MDA and H_2O_2 contents) in plants has been reported by Jday et al. (2016) and Nahar et al. (2017). Interestingly, NO treatment reduced the oxidative damage which is obvious by reduction of MDA and H_2O_2 content. NO-induced oxidative stress alleviation under drought stress was also observed in maize (Yildiztugay et al. 2014) and sunflower (Cechin et al. 2015).

Oxidative stress is not a sudden phenomenon because ROS level is tightly controlled at a level required for cellular signaling, growth, and metabolism (Cruz de Carvalho 2008; Hasanuzzaman et al. 2017a). ROS level is mainly controlled in the cell by antioxidant defense system (Gill and Tuteja 2010; Foyer and Noctor 2011). Therefore, we further checked the role of NO in regulating the antioxidant defense mechanism by measuring nonenzymatic antioxidant and antioxidant enzyme activity. Three enzymes namely CAT, GPX, and APX can detoxify H_2O_2 into water, whereas APX requires AsA for catalyzing this reaction (Ahmad et al. 2014; Nahar et al. 2015a). In our experiment, CAT activity decreased, and GPX activity increased under both levels of drought stress, whereas APX activity decreased only at severe drought stress created by 20% PEG (Figures 4(a) and 5(b,c)). Though GPX activity increased, it could not be able to reduce the H_2O_2 content alone. For this reason, higher amount of H_2O_2 was recorded in rapeseed seedlings under the 20% PEG-induced drought stress (Figure 2(b)). Drought-induced CAT activity reduction was observed by Nahar et al. (2015b) in mung beans, APX activity reduction by Xu et al. (2011) in Kentucky bluegrass, and GPX activity increase was observed in *Brassica napus* by Alam et al. (2014). NO treatment increased the CAT, APX, as well as GPX activity under drought, which consequently lowered the H_2O_2 content in drought-stressed plants. During H_2O_2 detoxification, MDHA is produced along with water. MDHA is partly converted to DHA by disproportionation reaction. Then both MDHA and DHA are used in the regeneration of AsA by MDHAR and DHAR enzymes (Gill and Tuteja 2010). As 2011; Akram et al. 2017). Therefore, increase in AsA content at mild drought and decrease in Figures 3(a) and 4(b)). GSH is another strong nonenzymatic antioxidant present in the antioxidant defense system playing a role in diverse metabolic function (Hasanuzzaman, Nahar, Anee et al. 2017a). GSH is a substrate for GPX and GST and is involved in AsA regeneration and glyoxalase system (Hasanuzzaman, Nahar, Anee et al. 2017a). The level

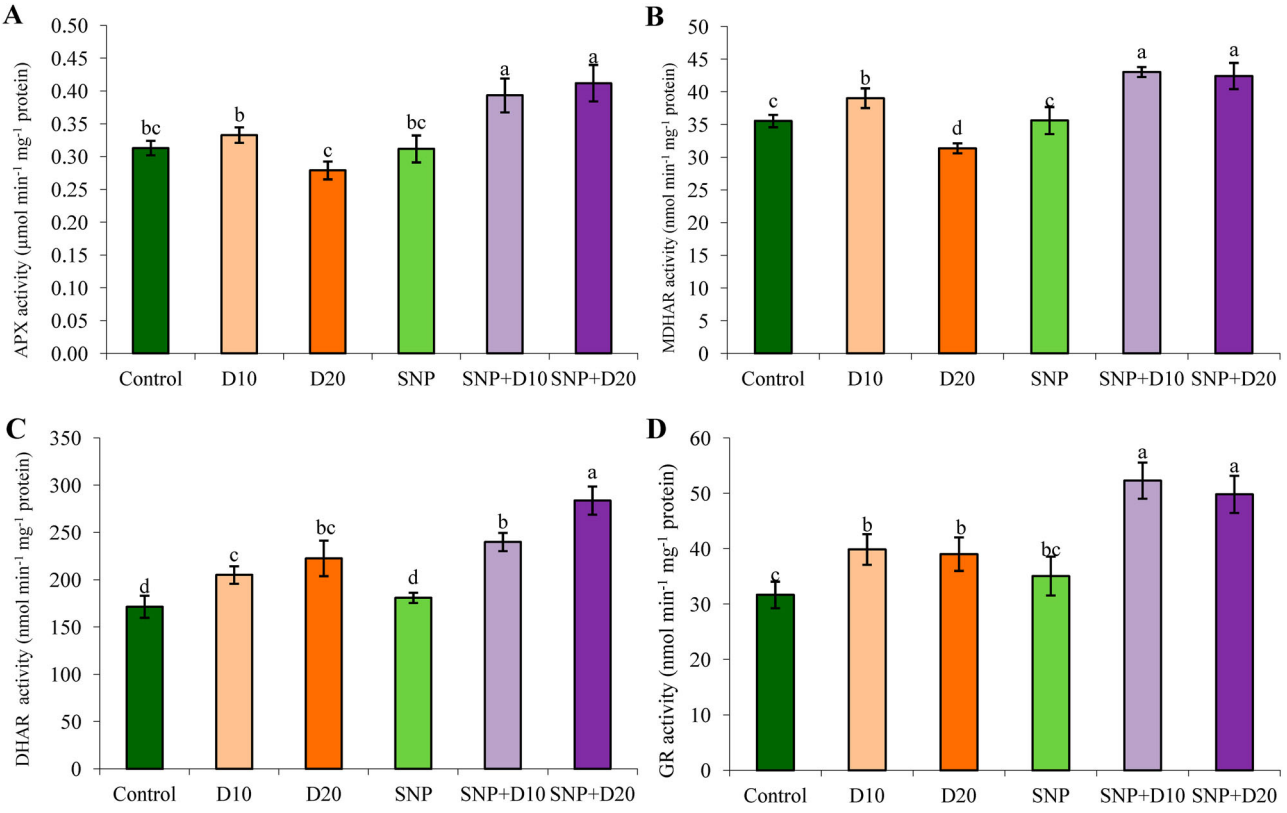

Figure 4. NO donor, SNP-induced changes in APX activity (A), MDHAR activity (B), DHAR activity (C), and GR activity (D) under drought stress. Treatments: Control, seedlings treated with nutrient solution only; D10, seedlings treated with 10% PEG; D20, seedlings treated with 20% PEG; SNP, seedling treated with 500 μM SNP for 24 h; SNP + D10, 500 μM SNP-pretreated seedlings exposed to 10% PEG; SNP + D20, 500 μM SNP-pretreated seedlings exposed to 20% PEG. Mean (±SD) was calculated from three replicates for each treatment. Vertical bars with different letters are significantly different at $P \leq .05$, determined by Fisher's LSD test.

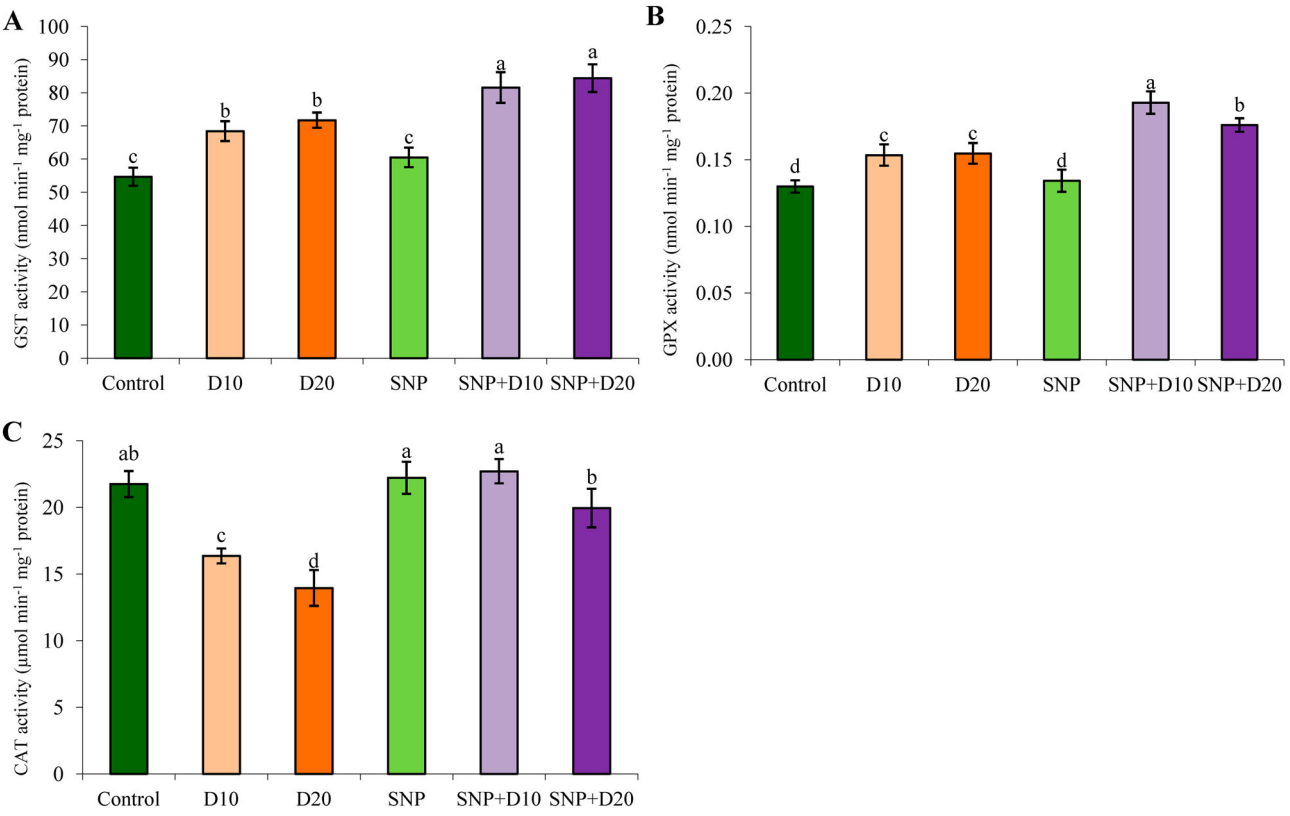

Figure 5. NO donor, SNP-induced changes in GST activity (A), GPX activity (B), and CAT activity (C) under drought stress. Treatments: Control, seedlings treated with nutrient solution only; D10, seedlings treated with 10% PEG; D20, seedlings treated with 20% PEG; SNP, seedlings treated with 500 μM SNP for 24 h; SNP + D10, 500 μM SNP-pretreated seedlings exposed to 10% PEG; SNP + D20, 500 μM SNP-pretreated seedlings exposed to 20% PEG. Mean (±SD) was calculated from three replicates for each treatment. Vertical bars with different letters are significantly different at $P \leq 0.05$, determined by Fisher's LSD test.

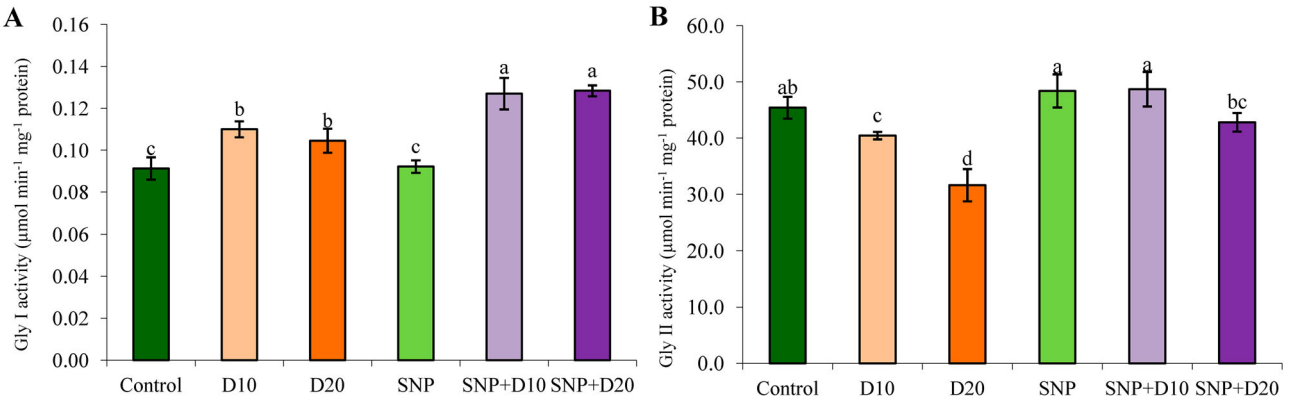

Figure 6. NO donor, SNP-induced changes in Gly I activity (A) and Gly II activity (B) under drought stress. Treatments: Control, seedlings treated with nutrient solution only; D10, seedlings treated with 10% PEG; D20, seedlings treated with 20% PEG; SNP, seedling treated with 500 μM SNP for 24 h; SNP + D10, 500 μM SNP-pretreated seedlings exposed to 10% PEG; SNP + D20, 500 μM SNP-pretreated seedlings exposed to 20% PEG. Mean (±SD) was calculated from three replicates for each treatment. Vertical bars with different letters are significantly different at $P \leq .05$, determined by Fisher's LSD test.

of GSH in cellular organelles is mostly determined by GR activity. Higher activity of GR was the reason for increased GSH content under drought stress (Figures 3(b) and 4(d)). Similar results were observed in maize shoot and root under drought stress by Ahmad et al. (2016a). Surprisingly, exogenous application of NO further increased the GR activity as well as the GSH content under both levels of drought stress (Figures 3(b) and 4(d)). The cellular redox signaling also depends on GSH and GSSG ration that regulates the cell cycle, gene expression, and protein function under favorable and adverse conditions (Szalai et al. 2009; Nahar et al. 2016). Under drought, GSSG increased in a dose-dependent manner, possibly due to the upregulation of DHAR, GPX, and GST under drought. Consequently, GSH/GSSG decreased. SNP supplementation improved the ratio by increasing GSH content. GST is a diverse gene family that can detoxify peroxides using GSH as a substrate (Nahar et al. 2016). In our experiment, GST activity increased under drought stress and further increased due to SNP, implying a positive role of GST in ROS metabolism. Upregulation of antioxidant enzymes confers abiotic stress tolerance by maintaining ROS below a threshold level (Gill and Tuteja 2010; Ahmad et al. 2014). Therefore, in this study, drought tolerance in rapeseed seedlings might be associated with NO-induced upregulation of antioxidant enzymes. NO can act as an ROS scavenger as well as a signaling molecule that enhances the expression of antioxidant enzymes (Groß et al. 2013). Furthermore, exogenous application of SNP might increase the endogenous NO to a level required for activation of antioxidant genes (Xu et al. 2010; Fan and Liu 2012; Ahmad et al. 2016b). The role of NO is not limited to stimulate antioxidant defense genes; it can increase the GSH content in the cell (Kovacs et al. 2015). Thus, in this experiment, lower oxidative damage in SNP-treated rapeseed seedlings is associated with NO-induced upregulation of antioxidant enzymes and increased level of GSH.

Glyoxalase system is composed of two enzymes, Gly I and Gly II, to detoxify MG when overproduced under abiotic stress conditions (Hasanuzzaman, Nahar, Hossain et al. 2017b). Thus upregulation of these enzymes is expected to enhance MG detoxification. Gly I activity slightly increased and Gly II activity decreased under drought stress (Figure 6). Similar results were reported by Nahar et al. (2017) in mung beans. However, both enzymes; activities increased under drought stress after treated with SNP, indicating the role of NO in drought stress tolerance by enhancing glyoxalase system (Figure 6). NO-induced upregulation of Gly I and Gly II was reported in rapeseed seedlings under salinity stress (Hasanuzzaman et al. 2011) which is in agreement with our experiment.

Conclusion

In this study, exogenous NO exhibited its protective effect against drought-induced damages in rapeseed plants which were attributed to regulation and improvement of water status; enhancement of antioxidant defense mechanism and relaxation of oxidative stress; upregulation of MG detoxification system; and thus alleviation of the toxic effects of MG. In spite of clear evidence of NO-induced advantageous effects on some biochemical and physiological parameters in rapeseed plant, this study demands advanced comprehensive study to explicate the status of NO synthesis inside the plant or the possible signaling pathways through which NO was successful in osmoregulation, was able to improve antioxidant defense, and MG detoxification system. Did NO affect/enhance the biosynthesis of metabolites/antioxidant molecules/enzymes or it prevented their degradation under drought stress? Cross-talk of NO with other molecules cannot be avoided too. Disclosing these aspects will make NO a more promising and defending molecule against abiotic stresses.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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