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Combined hydrogen peroxide and nitric oxide priming modulate salt stress tolerance in acclimated and non-acclimated oilseed rape (*Brassica napus* L.) plants

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Abstract

We examined the combined effects of hydrogen peroxide (H_2O_2) and nitric oxide (NO) on the responses of oilseed rape (*Brassica napus* L.) plants to salt stress under acclimated and non-acclimated conditions. The results of the shoot and root dry weight traits together with the measurement of malondialdehyde (MDA) indicated that salt acclimation with a low concentration of NaCl (50 mM) could not alleviate the inhibitory effect of high salinity (200 mM NaCl). Under acclimated conditions, seed priming with H_2O_2 or NO resulted in effective protection against salt stress, however, maximum amelioration of salt stress was found by the combined treatments of H_2O_2 + NO. Interestingly, in the salt-exposed non-acclimated plants, only seed priming with H_2O_2 + NO was effective in improving salt tolerance. Pretreatment with H_2O_2 + NO tended to limit Na translocation into photosynthetic organs to prevent salt damages. Additionally, a large increase in salicylic acid content was correlated with phenylalanine ammonia lyase activation and flavonoid biosynthesis was observed when oilseed rape plants exposed to salinity in the presence of H_2O_2+NO. Interestingly, in this study, endogenous NO content of H_2O_2–primed plants exhibited a significant increase under non-saline conditions, indicating that H_2O_2 influences NO accumulation. In addition, oilseed rape plants primed with H_2O_2 + NO exhibited lower MDA and H_2O_2 content, contributing to the better induction of antioxidative enzyme activities. Higher levels of antioxidant enzyme activities maintained the integrity of cell membranes, resulting in better plant growth under salt stress. Taken together, our results revealed that oilseed rape plants pretreated with H_2O_2 + NO exhibited more effective tolerance to salt stress than plants that were pretreated with H_2O_2 or NO alone.

Keywords: Combined priming; Flavonoid; Ion homeostasis; Nitric oxide; Oilseed rape; Salicylic acid; Salinity

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Introduction

Salinity stress induces osmotic stress and ion toxicity in plants, which mainly results in oxidative damage due to the production of reactive oxygen species (ROS) (Munns and Tester 2008). Overproduction of ROS ultimately causes membrane damage, which decreases the photosynthetic capacity. Therefore, to protect cells against ROS stress, plants possess several defensive strategies including the stimulation of antioxidant enzymes (Rajabi *et al.* 2012; Farzane *et al.* 2020), the accumulation of compatible solutes (Geranpayeh *et al.* 2017), the alteration in the compartmentalization of ions and the adjustment of photosynthetic processes (Parida and Das 2005).

It has been reported that salt tolerance may be improved by the exposure of plants to the low concentration of salt and subsequent higher levels (Pandolfi *et al.* 2016). Furthermore, the priming of plants by chemical compounds has been proposed as an effective method in increasing tolerance to different abiotic stresses. Hydrogen sulphide (H_2S) (Li *et al.* 2013), proline (Islam *et al.* 2009), hormones (Li *et al.* 2014), sodium nitroprusside (SNP), H_2O_2, melatonin (Mel) and polyamines (PAs) (Savvides *et al.* 2016) have been used as the priming agents against abiotic stresses. Some studies have suggested that the initial exposure to H_2O_2 and/or NO at an appropriate level can improve abiotic stress tolerance in plants (Habibi 2019). Several research reports indicated that exogenously applied H_2O_2 increased salt tolerance of wheat (Li *et al.* 2011), maize (Gondim *et al.* 2012, 2013), *Suaeda fruticosa* (Hameed *et al.* 2012) and *Panax ginseng* (Sathiyaraj *et al.* 2014) seedlings through stimulation of enzymatic and non-enzymatic antioxidants, and by increasing proline, polyamines and soluble sugars in maize seedlings under osmotic stress (Terzi *et al*. 2014). Additionally, exogenous application of NO increases salt tolerance by improving osmolyte accumulation, ionic homeostasis and antioxidative defense system (Hayat *et al.* 2012; Khan *et al.* 2012; Tanou *et al.* 2012; Egbichi *et al.* 2014), and by increasing the contents of ascorbic acid (AsA), proline and total phenolic under salinity conditions (Ali *et al.* 2017).

Since the yield of oilseed rape (*Brassica napus* L.) markedly decreases by salinity, improving salt stress tolerance in this species is important to improve crop productivity for the growing world population. As mentioned above, it has been indicated that appropriate levels of exogenous H_2O_2 and NO, applied through seed priming, are beneficial to mitigate salt stress, however, few studies have tested the combined effect of H_2O_2 with NO on salt-exposed plants. In this study, we hypothesized that oilseed rape plants primed with H_2O_2 + NO will show more effective tolerance to salt stress than plants that are pretreated with H_2O_2 or NO alone. Furthermore, up to date, no study has focused on the roles of H_2O_2 + NO in the acclimated plants under salt stress conditions. Therefore, we studied the exogenous H_2O_2 and/or NO that can mitigate oxidative damage in the salt acclimated oilseed rape plants against subsequent salt stress.

Materials and Methods

Plant materials and treatments

Seeds of oilseed rape (*Brassica napus* L. cv. Gabriella) were sown on the cylindrical plastic pots. For the priming treatments, oilseed rape seeds were soaked in aerated solutions of SNP (as the NO donor) and H_2O_2 for 12 h. The concentrations of NO and H_2O_2 were chosen according to the effect of different NO (10, 100, 500, 1000, 1500 and 2000 µM SNP) and H_2O_2 (1, 10, 20, 30, 40, 50, 100 and 200 mM H_2O_2) concentrations on oilseed rape seed germination as well as on seedling growth in a preliminary study, which exhibited that 1000 µM NO and 20 mM H_2O_2 priming significantly promoted seed germination and seedling growth (data not shown). Four seeds were planted in each pot (filled with sandy loam soil; pH 7.2), however, the seedlings were thinned to one plant per pot after emergence. Pots had a diameter of 14 cm and a depth of 45 cm. To maintain the 90% field capacity, the seedlings were irrigated with distilled water every five days. Nine weeks after sowing, when the plants were about 12 cm tall, the pots were irrigated with 50 mM NaCl solution dissolved in tap water for seven days (ACC; salt acclimation). To impose salt stress, saline solutions of 0.2-1.0 g NaCl were added to the pots based on Hajiboland *et al.* (2010). A regression equation was used to determine the amount of NaCl for obtaining different salinity levels. After the acclimation period, the plants were irrigated with 200 mM NaCl (salt stress). Acclimation treatments were as follows: control (non-acclimated, non-stressed), ST (non-acclimated, stressed) and ACC+ST (acclimated, stressed). To obtain similar sizes of acclimated and not-acclimated plants at the beginning of the salinity treatment, the level of salinity concentration for the initial acclimation experiment was selected based on Pandolfi *et al.* (2016). The EC for control pots was about 1.28 dS m^{-1}. Throughout the experiment, the day/night temperature was 25-30/19-21 ºC, relative humidity was 60-65 % and the daily photon flux density was about 350-400 µmol m^{-2} s^{-1}.

Harvest

Enzymes were measured on the fully expanded leaves after two weeks of treatment with 200 mM NaCl. Shoots and roots were separated, washed with distilled water, dried on filter paper and then the fresh weight was measured. To determine the dry weight of the hoots and roots they were dried at 70 °C for 48 h. The leaf samples were frozen immediately in liquid N_2 and stored for further assays.

Determination of proline, soluble proteins, soluble sugars and starch

Proline was determined according to the method of Bates *et al.* (1973). The total soluble proteins were measured as described by Bradford (1976) utilizing a commercial reagent (Sigma) and BSA (Merck) as the standard. Soluble sugars were measured based on Quentin *et al.* (2015). Starch was determined using the method of Magne *et al.* (2006).

Assay of phenylalanine ammonia-lyase (PAL) activity and related metabolites

Leaf samples were ground in 50 mM sodium phosphate buffer (pH 7.0) containing 2% (w/v) polyvinylpolypyrrolidon (PVPP), 18 mM β-mercaptoetanol, 0.1% (v/v) triton X-100 and 2 mM EDTA. The modified method of Zucker (1965) was utilized to determine the formation of cinnamic acid by spectrophotometry at 290 nm. Total flavonoid content and total phenolic content were measured using the methods of Meda *et al.* (2005) and Velioglu *et al.* (1998), respectively.

Determination of Na and K content

All samples were dried at 550 °C for 8 h and the dry powders were extracted with 0.5 M HCl and made up to volume by the double-distilled water. The resulting solution was diluted in distilled water, and the Na and K concentrations were measured by an Inductively-Coupled Plasma-Atomic Emission Spectrometry (ICP-AES, INTEGRA XL2, GBC; Australia).

Assay of antioxidative enzymes and related metabolites

The activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were measured according to methods described elsewhere (Habibi and Hajiboland 2012). Nitrate reductase (NR, EC 1.7.99.4) activity was evaluated by measuring the amount of nitrite formed in the NR assay buffer according to the method of Robin (1979). NR activity was expressed as µmol nitrite g^{-1} FW h^{-1}. MDA levels were quantified from a 1, 1, 3, 3-tetraethoxypropane (Sigma) standard curve. The hydrogen peroxide (H_2O_2) content in the leaves was evaluated according to the method of Velikova *et al.* (2000). The H_2O_2 content was given on a standard curve.

Quantification of NO concentration

Nitric oxide concentration was determined according to Wu *et al.* (2016). Absorbance was monitored at 540 nm. The NO concentration was calculated using a standard curve plotted with known concentrations of $NaNO_2$.

HPLC analysis

Extraction and determination of SA were done according to Kadioglu *et al.* (2011). The leaves (0.5 g) were extracted with a fixative solution containing methanol (3 ml) at room temperature. Subsequently, it was centrifuged at 12,000g for 15 min and then filtered. The calibration curve was performed using methanol to obtain a 1 mg/ml concentration, and the areas of peaks of the compound were quantified by the Agilent Chem Station software. The HPLC analysis was performed on Hichrom C_{18} column (5µ, 250 mm) at 290.4 nm using Agilent 1100 series system. For the chromatographic assay separation, the total running time was 10 minutes at a flow rate of 1 ml/min.

Statistical analysis

The experiment was carried out as factorial using a completely randomized design with four independent replications. All data were subjected to one-way analysis of variance and significant differences between means were determined by the Tukey's test ($p \leq 0.05$) using the Sigma Stat (3.5) software.

Results

Under non-saline conditions, H_2O_2-primed and H_2O_2+NO-primed plants had significantly higher shoot dry weight than the non-primed plants (Figure 1A). The shoot dry weight was decreased by ACC+ST or the single ST treatment. Interestingly, priming seeds with H_2O_2, NO, or H_2O_2+NO alleviated the deleterious effects of ACC+ST or ST stress on the shoot growth. However, the shoot dry weight was enhanced only by H_2O_2+NO priming under control conditions. The root dry weight was enhanced to a higher extent in response to H_2O_2 priming than other treatments (Figure 1B). The root dry weight of plants presented a significant decrease in response to ACC+ST or ST treatment, except for H_2O_2+NO-primed plants under ACC+ST. Under ST conditions, while root dry weight was improved by H_2O_2+NO priming, it was not affected by exposure to H_2O_2 or NO priming alone, compared with the control.

Both shoot and root Na contents were increased by the ACC+ST treatment and further increased by the ST treatment (Figure 2). Under ST conditions, while shoot and root Na content was not affected by the exposure to H_2O_2 or NO alone, it was significantly decreased by the combination of H_2O_2 and NO. Shoot and root K

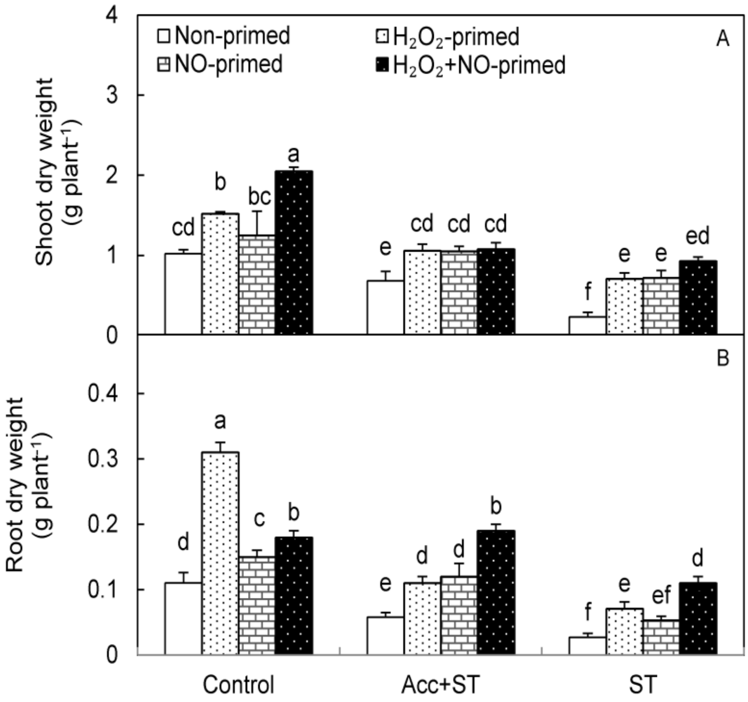

Figure 1. Effects of salt stress on the shoot and root dry weight of oilseed rape plants primed with hydrogen peroxide and nitric oxide under acclimated (ACC+ST) and non-acclimated (ST) conditions. Bars indicated with the same letter are not significantly different ($p \leq 0.05$, Tukey's test). Values are the mean ± SD (n= 4).

contents were significantly decreased by ACC+ST and ST treatments, as compared to non-salt treatment (Figure 2). However, this decrease in K concentration was slightly prevented in ACC + ST treatment by H_2O_2+NO priming.

Results indicated that priming with H_2O_2 and NO increased soluble sugars while decreasing starch, and further increases were observed in soluble sugars concentrations in plants grown under salt stress (Table 1). Under non-saline conditions, no significant differences in proline content were mainly detected among plants grown with or without the priming agent. However, the highest values of proline contents were observed in salt-exposed acclimated or non-acclimated plants as compared to control.

Under non-saline conditions, phenolic and flavonoids contents and PAL activity were not affected by exposure to H_2O_2 and NO priming (Figure 3). However, phenolic and flavonoids contents were significantly increased by H_2O_2, NO and H_2O_2+NO priming under ACC+ST conditions. Under ST conditions, while the phenol and flavonoids contents, as well as the activity of PAL enzymes, were improved by the H_2O_2+NO priming, they were not affected by exposure to H_2O_2 or NO priming alone, compared with control.

The SOD activity was significantly increased by salt stress, and a further increase was observed under combined salt and priming treatments (Figure 4). A similar observation was recorded for

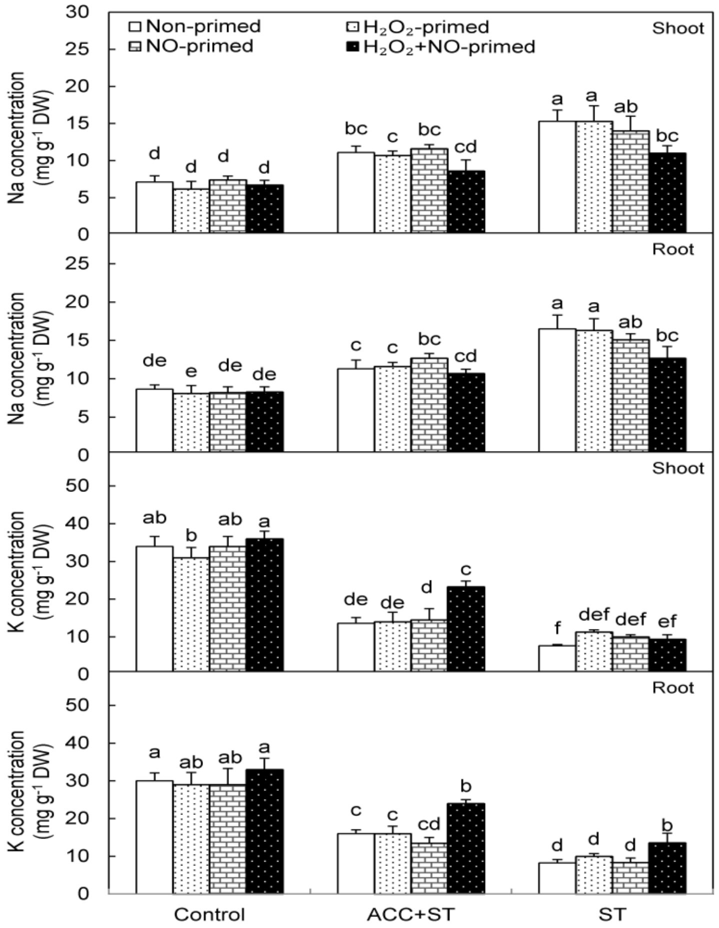

Figure 2**.** Effects of salt stress on the shoot and root Na and K content of oilseed rape plants primed with hydrogen peroxide and nitric oxide under acclimated (ACC+ST) and non-acclimated (ST) conditions. Bars indicated with the same letter are not significantly different ($p \leq 0.05$, Tukey's test). Values are the mean ± SD (n= 4).

CAT and POD activity patterns in plants exposed to salt and combined priming. The NR activity in the non-primed salt-stressed plants remained unchanged but enhanced in response to NO or H_2O_2+NO priming under both saline and non-saline conditions.

Both MDA and H_2O_2 contents were significantly enhanced by the ACC+ST treatment and further increased by the ST treatment, whereas the priming with H_2O_2, NO, or H_2O_2+NO reduced the content of these oxidants under salt stress (Figure 5). Furthermore, under ST conditions, H_2O_2+NO-pretreated plants exhibited the most dramatic decreases in MDA and H_2O_2 contents than plants that were primed with H_2O_2 or NO alone.

The results indicated that both SA and NO contents were significantly improved by the priming with H_2O_2, NO, or H_2O_2+NO under non-saline conditions (Figure 6). Although higher leaf SA and NO content were detected in the salt-acclimated plants, the plants under the combination of salt and priming treatments had the highest SA content (Figure 6). Oilseed rape plants primed with H_2O_2+NO showed higher SA and NO content under both ST and ACC+ST conditions than plants that were primed with H_2O_2 or NO alone.

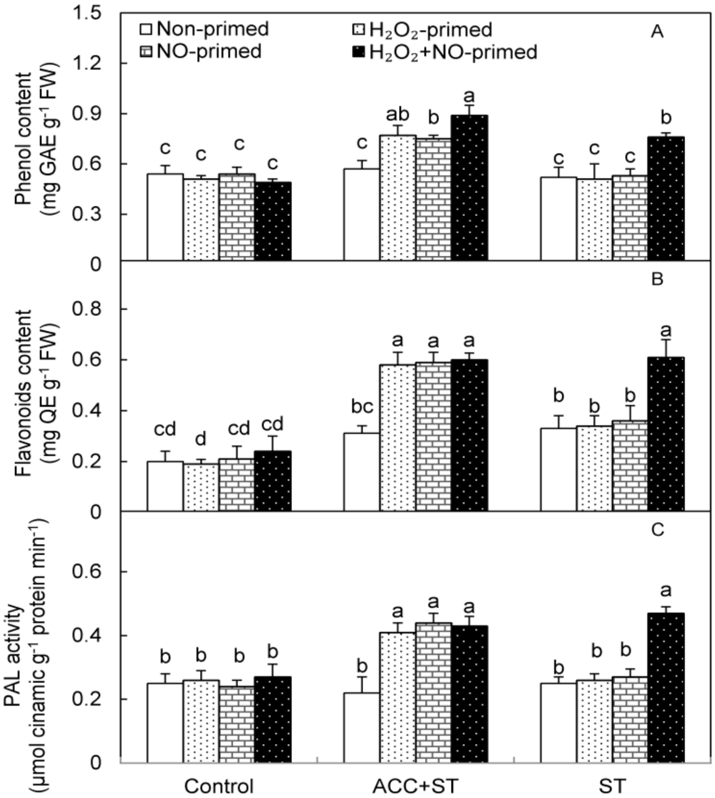

Figure 3. Effects of salt stress on the total phenol and flavonoids content and the activity of phenylalanine ammonia-lyase (PAL) in oilseed rape plants primed with hydrogen peroxide and nitric oxide under acclimated (ACC+ST) and non-acclimated (ST) conditions. Bars indicated with the same letter are not significantly different ($p \leq 0.05$, Tukey's test). Values are the mean ± SD (n= 4).

Discussion

Exogenous H_2O_2 and NO pretreatment improved growth of oilseed rape under salt stress

Oilseed rape is considered a moderately salt-sensitive crop, and its growth and yield are reduced at high salinity (Kholghi *et al.* 2018). In this study, shoot and root dry weight were significantly decreased in oilseed rape plants grown under ACC+ST and ST conditions. These observations were consistent with the findings of Kholghi *et al*. (2018), who showed that a high NaCl concentration (150 mM) significantly decreased the shoot and root dry weight of oilseed rape genotypes. However, the growth inhibition under ACC+ST was mitigated by H_2O_2 and NO priming. Similar results were obtained by Ali *et al.* (2017), who reported that the growth of wheat cultivars seedling was increased by SNP priming under salinity. Additionally, our results were consistent with the findings of Kilic and Kahraman (2016) for barley seedlings, who found that the growth of NaCl-treated plants was enhanced by seed priming with NO. In our study, oilseed rape plants pretreated with H_2O_2+NO exhibited better growth under ST conditions than plants that were pretreated with H_2O_2 or NO alone, suggesting that H_2O_2+NO application was more effective than H_2O_2 or NO alone for oilseed rape plants exposed to 200 mM NaCl stress.

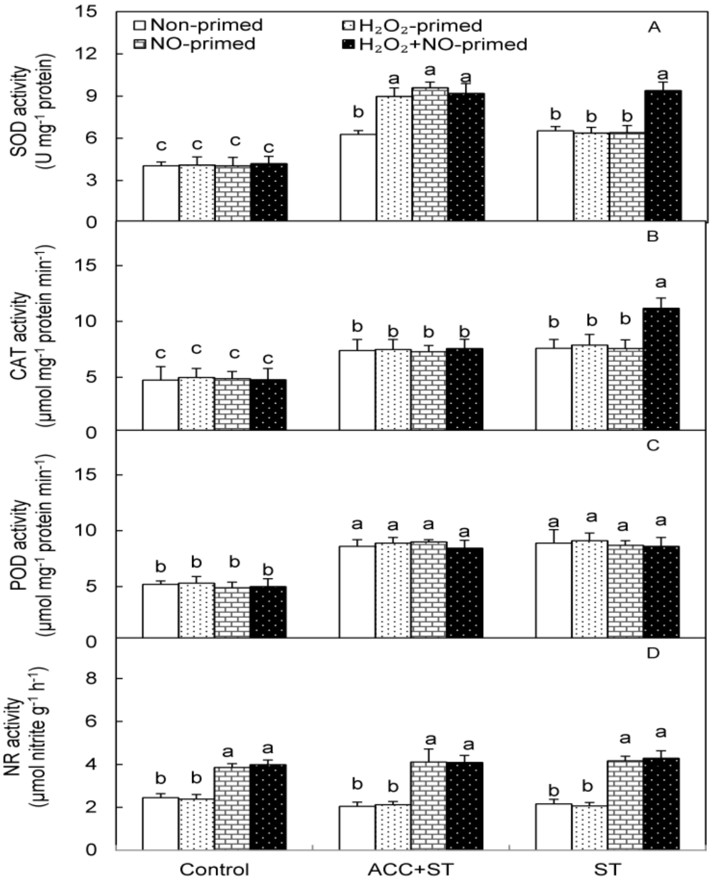

Figure 4. Effects of salt stress on the activity of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and nitrate reductase (NR) of oilseed rape plants primed with hydrogen peroxide and nitric oxide under acclimated (ACC+ST) and non-acclimated (ST) conditions. Bars indicated with the same letter are not significantly different ($p \leq 0.05$, Tukey's test). Values are the mean ± SD (n= 4).

Priming altered Na distribution in shoots under salt stress

Salt stress causes the accumulation of Na in the leaves and consequently, excessive Na-toxicity decreases plant productivity (Munns and Tester 2008; Habibi 2017; Jiang *et al.* 2017). This study showed that the shoot and root Na contents were increased under salt stress. However, seed priming with the combination of H_2O_2 and NO decreased Na in the shoots and roots while increasing K in the roots in oilseed rape plants grown under salt stress. The improvement of the K/Na ratio has also been reported in the salt-stressed wheat plants in response to H_2O_2 application (Ashfaque *et al.* 2014) and salt-stressed sunflower seedlings in response to NO priming (David *et al.* 2010). Therefore, these results further demonstrated the beneficial effects of H_2O_2+NO application in the salt-stressed oilseed rape plants, which tended to limit Na translocation into photosynthetic organs to protect them from salt damages.

Exogenous H_2O_2 and NO pretreatment altered compatible solute content under NaCl stress

Soluble sugars contents increased significantly under salt stress and priming treatments. The

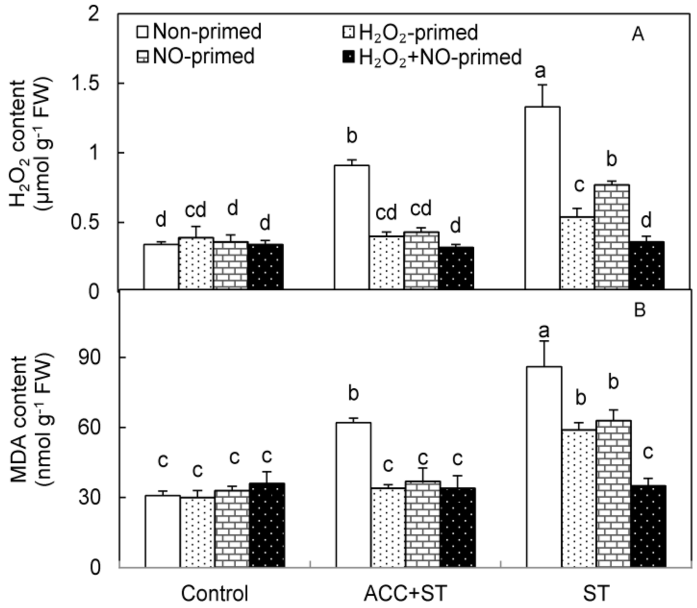

Figure 5. Effects of salt stress on the concentration of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in oilseed rape plants primed with hydrogen peroxide and nitric oxide under acclimated (ACC+ST) and non-acclimated (ST) conditions. Bars indicated with the same letter are not significantly different ($p \leq 0.05$, Tukey's test). Values are the mean ± SD (n= 4).

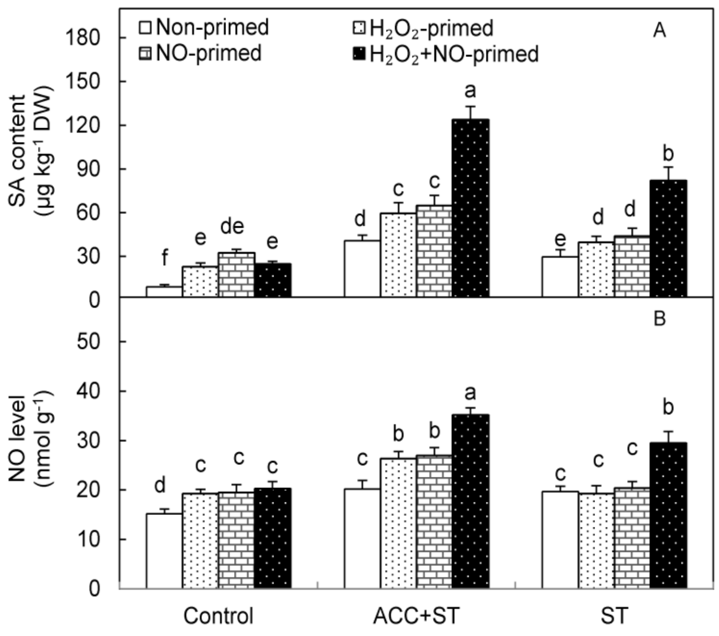

Figure 6. Effects of salt stress on endogenous salicylic acid (SA) and nitric oxide (NO) contents of oilseed rape plants primed with hydrogen peroxide and nitric oxide under acclimated (ACC+ST) and non-acclimated (ST) conditions. Bars indicated with the same letter are not significantly different ($p \leq 0.05$, Tukey's test). Values are the mean ± SD (n= 4).

improvement of soluble sugars and proline has also been reported in the salt-stressed maize seedlings in response to H_2O_2 application (Terzi *et al.* 2014). Many plants when exposed to salinity stress accumulate proline in large quantities as the ROS scavenger and osmotic regulator (Chun *et al.* 2018). In this study, we found that salinity at 200 mM led to high levels of proline, indicating that osmotic stress was occurred (Ma *et al.* 2016). Ellouzi *et al*. (2017) reported that H_2O_2 seed priming increased proline production in *Cakile maritima* and *Eutrema salsugineum* grown under salinity. Additionally, Ali *et al.* (2017) showed that priming of wheat seeds with SNP alleviated the adverse effect of salt stress by increasing the proline content. However, our results showed that the proline content was significantly increased by salt treatment, while it was not mainly affected by priming treatments in non-salt treated oilseed rape plants, suggesting that proline accumulation was not involved in H_2O_2- and NO-induced salt stress tolerance.

Priming enhanced phenolic accumulations under NaCl stress

Previously, several studies have indicated that phenolic compounds play important roles in radical scavenging as well as plant responses to salt stress (Oh *et al.* 2009; Mahmoudi *et al.* 2010; Blasco *et al.* 2013; Su *et al*. 2018). Similar to these studies, we found an increase in flavonoids content in salt-exposed non-acclimated plants. When salt-exposed acclimated oilseed rape plants were primed with H_2O_2, NO, or H_2O_2+NO, a further increase in phenolic and flavonoids contents as well as in PAL activity was observed. These results were in line with the findings of Ali *et al.* (2017) who showed a large increase in total phenolic content when wheat cultivars subjected to salinity in the presence of 0.1 mM SNP. Since carotenoids and flavonoids have been shown to modulate the ROS level and maintain photosynthetic capacity (Burchard *et al.* 2000; Xu and Rothstein 2018), we suppose that H_2O_2+NO mitigated the inhibitory effects of salt stress on the structure of the photosynthetic apparatus by increasing the total phenolic and flavonoids contents of oilseed rape leaves.

Priming stimulated the antioxidant defense systems during salt stress

It has been observed that antioxidant enzymes play important role in scavenging salt-induced plant ROS burst (Miller *et al*. 2010; Yang *et al.* 2014). In this study, the recorded elevations in antioxidant enzymes' activities were mainly higher in the primed salt-stressed plants in comparison with non-primed salt-stressed plants. Moreover, priming with H_2O_2+NO greatly reduced MDA and H_2O_2 contents, contributing to the better induction of antioxidative enzymes activities in response to salt stress. The present results are in agreement with the previous investigations, which revealed that pretreatment with H_2O_2 alleviated salt-induced inhibitory effects in *Suaeda fruticosa* (Hameed *et al.* 2012), *Panax ginseng* (Sathiyaraj *et al.* 2014), *Cakile maritima, Eutrema salsugineum* (Ellouzi *et al.* 2017), wheat (Li *et al.* 2011) and maize (Gondim *et al.* 2012, 2013) via modulation of the antioxidative mechanism involved in removing ROS. In addition, our results were consistent with the findings of Egbichi *et al.* (2014) for soybean and Ali *et al.* (2017) for wheat cultivars. They reported that NO alleviated salinity stress by enhancing the activities of antioxidant enzymes such as SOD, CAT and POD, which prevented oxidative damage. In the present study, the NR activity was significantly enhanced by single NO or by the combination of H_2O_2+NO under both saline and non-saline conditions. There are evidences reporting that application of NO stimulates the activities of nitrogen metabolism enzymes such as NR and nitrite reductase in tomato plants under salt-stress conditions (Manai *et al.* 2014) and wheat seedlings under non-saline conditions (Balotf *et al.* 2018). Interestingly, oilseed rape plants primed with H_2O_2+NO exhibited lower MDA and H_2O_2 content under ST conditions than plants that were primed with H_2O_2 or NO alone, showing that combined priming was more effective than H_2O_2 or NO alone in alleviating NaCl stress.

Priming increased the leaf NO and SA content under salt stress

In this study, the application of exogenous NO caused an increase in endogenous NO content compared with non-NO-treated plants. The increase in endogenous NO content following exogenous NO treatment was consistent with the findings of Balotf *et al.* (2018), which reported that exogenous NO caused an increase in leaf NO content in *Triticum aestivum* plants. Interestingly, in this study, endogenous NO content of H_2O_2–primed plants exhibited a significant increase under non-saline conditions, indicating that H_2O_2 influences NO accumulation. However, future work will need to understand which one is upstream of the other. In this study, the recorded increases in SA levels were higher in the primed salt-stressed plants in comparison with non-primed salt-stressed plants. This increase in SA levels may provide more stress tolerance. Additionally, our results demonstrated that a large increase in SA content was correlated with PAL activation when oilseed rape plants exposed to salinity in the presence of H_2O_2+NO. These observations were consistent with the findings of Li *et al.* (2019), which showed that SA increases the activity of PAL, which enhances flavonoid biosynthesis in tea leaves. Regarding relationships of SA and NO, Li *et al*. (2019) reported that SA acts upstream of NO to enhance flavonoid biosynthesis in tea plants under high CO_2 conditions. However, the involvement of SA in inducing NO content and how it regulates phenolic metabolism in the primed plants under salt-stress conditions are currently unknown.

Conclusion

Results showed that salt acclimation with a low concentration of NaCl (50 mM) could not mitigate the inhibitory effect of 200 mM NaCl. Under non-acclimated conditions, maximum amelioration of salt stress was found by only the H_2O_2+NO treatment. The increased salt tolerance in oilseed rape plants by seed priming with H_2O_2+NO was attributed to the enhanced compatible solute content and decreased Na^+ transport to the shoot, which could provide osmotic protection to cells. Our data indicated that exogenous H_2O_2+NO increased the SOD and CAT, and caused a large increase in SA content correlated with PAL activation and flavonoid (as non-enzymatic antioxidants) biosynthesis, resulting in better plant growth under salt stress (Figure 7). Regarding the effectiveness of H_2O_2 and NO priming and their combined treatments, the results exhibited that interplay among H_2O_2 and NO afforded more effective protection against salt stress. Some research reports have revealed that the interactions between H_2O_2 and NO are involved in physiological responses to various abiotic stresses (Huang *et al.* 2015; Shi *et al.* 2015 Niu and Liao 2016). Increasing evidence suggests that the relationship between H_2O_2 and NO may be involved in inducing tolerance to heat (Li *et al.* 2015; Wang *et al.* 2014), cold (Guo *et al.* 2014; Tan *et al.* 2013) and UV-B stress (Tossi *et al.* 2014). Several studies on plants have demonstrated that the crosstalk among H_2O_2 and NO signaling regulates plant responses to high salinity (Tanou *et al.* 2009, 2010; Niu and Liao 2016). However, the effects of multiple priming on photosynthesis and antioxidant machinery of the salt-stressed plants will need to be further analyzed.

Conflict of interest

The authors declare no conflict of interest.

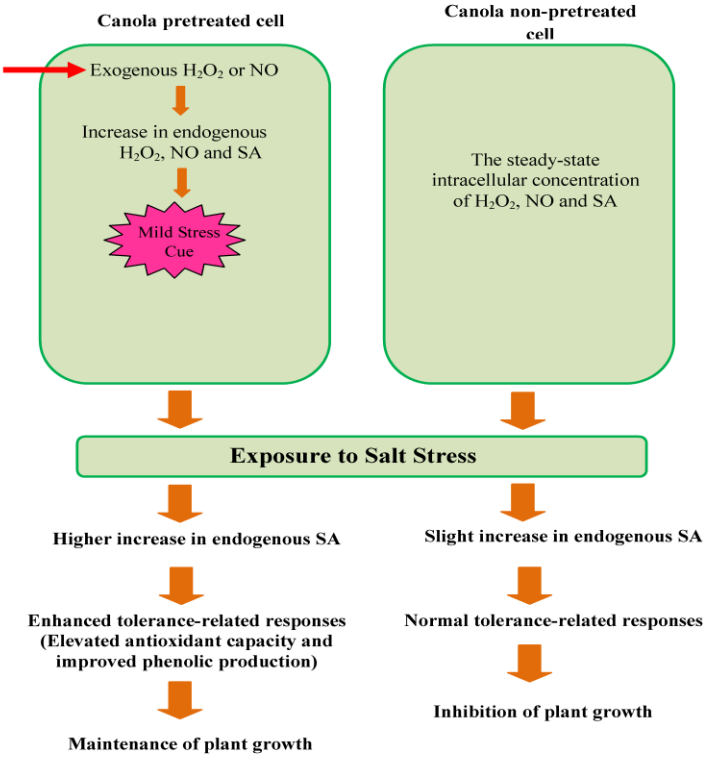

Figure 7. Priming with exogenous H_2O_2 and NO induces salt-stress tolerance of oilseed rape plants and improves physiological homeostasis and plant growth.

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بهبود تحمل به شوری در گیاه کلزا توسط پرایمینگ ترکیبی پراکسید هیدروژن و اکسید نیتریک در شرایط عادت‌دهی و بدون عادت‌دهی به شوری

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چکیده

در این پژوهش، تأثیر پرایمینگ بذور با پراکسید هیدروژن (H_2O_2)، اکسید نیتریک (NO) یا ترکیب H_2O_2+NO بر پاسخ های گیاه کلزا به شوری در شرایط عادت‌دهی و بدون عادت‌دهی به شوری مورد مطالعه قرار گرفت. نتایج حاصل از سنجش صفات وزن خشک ساقه و ریشه به همراه سنجش مالون دی آلدئید (MDA) نشان دادند که کاربرد غلظت اندک کلرید سدیم (۵۰ میلی مولار) به عنوان عامل عادت‌دهی به شوری، نتوانست باعث تخفیف اثرات زیانبار شوری بالا (۲۰۰ میلی مولار) شود. در عین حال، در شرایط عادت‌دهی، پرایمینگ بذور با H_2O_2 یا NO باعث تخفیف اثرات تنش شوری بر رشد کلزا شد، هرچند بیشترین تخفیف توسط ترکیب H_2O_2+NO به دست آمد. جالب توجه این که در گیاهان بدون عادت‌دهی به شوری، تنها پرایمینگ ترکیبی بذور با H_2O_2+NO در بهبود تحمل به شوری موثر بود. این تخفیف تنش توسط پرایمینگ ترکیبی در شرایط شوری، با کاهش انتقال سدیم به اندام هوایی، افزایش مقدار اسید سالیسیلیک، فلاوونوئیدها و فعالیت آنزیم فنیل آلانین آمونیالیاز همراه بود. پرایمینگ بذور با پراکسید هیدروژن در شرایط غیرشور، باعث افزایش مقدار NO داخلی شد. همچنین، پرایمینگ بذور با ترکیب H_2O_2+NO از طریق کاهش MDA و H_2O_2 داخلی و افزایش فعالیت آنزیم‌های سیستم آنتی اکسیدان، باعث حفظ تمامیت غشاها و در نتیجه بهبود رشد در شرایط شوری گردید. نتایج این تحقیق نشان داد که پرایمینگ ترکیبی بهتر از پرایمینگ تنها توانست اثرات زیانبار شوری در گیاه کلزا را تخفیف دهد.

واژه‌های کلیدی: اسید سالیسیلیک؛ اکسید نیتریک؛ پرایمینگ ترکیبی؛ شوری؛ فلاوونوئید؛ کلزا؛ هومئوستازی یونی