Botanica
ISSN 2538-8657
https://doi.org/10.35513/Botlit.2025.4.2
2025, 31(4): 155–175

Original research

Activation of triticale grain germination under high temperature and simulated drought by nitric oxide donor and its relationship with carbohydrate metabolism and resistance to oxidative stress

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Received: 19 September 2025. Accepted: 27 October 2025. Published online: 11 November 2025

Abstract

Shakhov I.V., Yastreb T.O., Taraban D.A., Oboznyi A.I., Shkliarevskyi M.A., Ryabchun N.I., Kolupaev Yu.E., 2025: Activation of triticale grain germination under high temperature and simulated drought by nitric oxide donor and its relationship with carbohydrate metabolism and resistance to oxidative stress. – Botanica, 31(4): 155–175. https://doi.org/10.35513/Botlit.2025.4.2

Triticale (× *Triticosecale* Wittm. ex A. Camus), an intergeneric hybrid of wheat and rye, is a valuable food and fodder crop. However, the germination of triticale seeds is strongly reduced under unfavourable conditions such as drought. One of the methods of increasing seed germination and activating plant growth in the early stages is seed priming with physiologically active substances, including donors of nitric oxide (NO) gasotransmitter. The effect of exogenous NO on the germination of triticale seeds has not yet been studied. This study aimed to investigate the impact of the nitric oxide donor sodium nitroprusside on seed germination and growth of triticale (× *Triticosecale* 'Raritet') seedlings under the action of high temperature (35°C) or drought simulation (12% PEG 6000, polyethylene glycol solution with a molecular weight of 6000 Da). Seed treatment with 100 µM sodium nitroprusside solution increased the germination index, seed germination percentage, seedling biomass, and vigour index under stress factors. Additionally, seed priming with sodium nitroprusside increased the water content of seedling tissues under PEG-simulated drought conditions. Exposure of seeds to sodium nitroprusside enhanced the generation of endogenous NO in shoots under stress conditions. In sodium nitroprusside-treated grains, there was an increase in amylase activity, and the sugar content increased in the shoots of seedlings under the action of high temperature and drought. In addition, sodium nitroprusside treatment promoted the preservation of a pool of anthocyanins with high antioxidant activity in the shoots under drought conditions. Pretreatment of seeds with sodium nitroprusside significantly reduced the accumulation of oxidative stress markers, namely hydrogen peroxide and lipid peroxidation product malondialdehyde, in shoots, caused by adverse influences. All the described effects of sodium nitroprusside were eliminated when seeds were treated with the nitric oxide scavenger methylene blue, indicating the specificity of sodium nitroprusside action as an NO donor. It was concluded that the most critical components of the effect of exogenous NO on seed germination and seedling growth at high temperatures and under drought conditions are the improvement of osmoregulation due to the accumulation of sugars and the prevention of oxidative damage development.

Keywords: carbohydrate metabolism, drought, high temperature stress, nitric oxide, resistance, seed germination, seedling growth, × *Triticosecale*.

INTRODUCTION

The period between seed sowing and seedling emergence plays a critical role in yield formation in most crops (Finch-Savage & Bassel, 2016). Low and irregular seed germination, which varies depending on weather conditions, can severely limit the yield of cultivated plants (Rhaman, 2025). Climate change and an increased incidence of abiotic stresses negatively impact seed germination and the early stages of seedling development, posing a significant challenge to global agriculture and food security (Das & Biswas, 2022). Cultivated plants, particularly winter cereals, are exposed to numerous abiotic stresses during the earliest stages of development. In recent decades, autumn droughts, often accompanied by high temperatures, have significantly impacted seed germination and seedling development (Romanenko et al., 2018).

To improve seed germination and seedling development under unfavourable conditions, various techniques have been developed over the past two decades, often becoming effective agricultural technologies. Among them, seed priming is a pre-sowing treatment in which seeds are moistened under controlled conditions to trigger key metabolic processes required for germination (Rhaman, 2025). It is well known that priming creates conditions for the initiation of pre-germinative processes in grains, which are physiological, biochemical, and molecular events that enhance the ability of seeds to germinate rapidly and uniformly even under stress conditions (Jatana et al., 2024). These include increased activity of enzymes involved in mobilising grain reserve substances (amylase, proteases), changes in hormonal regulation, and activation of the antioxidant system and repair mechanisms for oxidative stress-induced damage (Paparella et al., 2015; Monajjem et al., 2023; Rhaman, 2025). Primed seeds tend to show better adaptability under a wider range of abiotic and biotic stress conditions compared to non-primed seeds (Mustafa et al., 2017).

In recent years, the range of methods related to seed treatment with physiologically active substances, such as various plant hormones, stress metabolites (including antioxidants), and signalling compounds, has expanded alongside traditional methods of seed priming, including hydropriming, osmopriming, and thermopriming (Ashraf et al., 2019). Gasotransmitters, which are gaseous signalling molecules involved in regulating the cell cycle and processes such as seed germination and rhizogenesis (Kolupaev et al., 2022c; Verma & Prasad, 2023), as well as plant adaptation to adverse environmental conditions (Mazahar & Raina, 2023; Dey et al., 2024), are of particular interest in this group of compounds.

The most extensively studied gasotransmitter is nitric oxide (NO), which is considered one of the most important components of the signalling networks of plant and animal cells (Asgher et al., 2017; Zhou et al., 2021). In plants, NO can be formed by reductive pathways (from nitrate/nitrite) or oxidative pathways (from L-arginine) (Hancock & Neill, 2019; Kolbert et al., 2019). Researchers have accumulated a considerable amount of data on the effect of nitric oxide on the seed germination of plants of different species (Zhao et al., 2020; Kolupaev et al., 2024a; Ullah et al., 2024).

The processes of seed dormancy release and germination initiation are known to be caused by increased water content in the axial parts of the embryo and changes in the balance of plant hormones, particularly abscisic acid, gibberellins, auxins, and ethylene. Cell cycle activation, which is essential for embryo germination, has been demonstrated to be induced by increased ethylene and gibberellin levels as well as decreased abscisic acid content (Kepczynski et al., 2017). Such changes can be induced by signalling mediators, particularly nitric oxide, even through exogenous applications (Liu et al., 2011). To date, the general molecular mechanisms by which nitric oxide affects the hormonal balance of germinating seeds have been elucidated (Arc et al., 2013; Kolupaev et al., 2024a). These effects are mainly due to post-translational modifications of target proteins (Jasid et al., 2008). In *Arabidopsis*, tyrosine nitration has been shown to inhibit the molybdenum cofactor of sulphurase, the enzyme in the final step of abscisic acid synthesis (Lozano-Juste et al., 2011). Inactivation of abscisic acid synthesis by this mechanism may promote seed germination (Rajjou et al., 2012). Additionally, NO induces S-nitrosation of several proteins involved in abscisic acid signalling and transduction, which inhibits this signal (Signorelli & Considine, 2018). At the same time, nitric oxide leads to the activation of gibberellin (Signorelli & Considine, 2018) and ethylene synthesis enzymes (Kolbert et al., 2019), which can activate germination. Seed treatment with

NO has been shown to directly alter the activity of key ethylene synthesis enzymes such as S-adenosyl-L-methionine methylthioadenosine-lyase and 1-aminocyclopropane-1-carboxylic acid oxidase (Gniazdowska et al., 2010). NO is also known to induce cell cycle gene expression and increase amino acid content required for synthesising new proteins in germinating seeds (Brouquisse, 2019). Another mechanism by which nitric oxide affects seed germination involves its role in regulating systems that generate reactive oxygen species. Moderate amounts of reactive oxygen species contribute to seed germination by activating abscisic acid degradation processes and stimulating the antioxidant system (Ciacka et al., 2022).

Treating seeds from plants of various taxonomic groups, including cereals, with nitric oxide donors has been shown to promote germination under adverse conditions and increase resistance to stressful temperatures and drought (Kaur & Kaur, 2018; Bibi et al., 2020; Ciacka et al., 2022).

Triticale, a hybrid species obtained by crossing wheat and rye, combines the properties of a food and a fodder crop. According to the results of field trials analysing different cultivars, triticale has been found to be more drought-tolerant than wheat species (Blum, 2014). However, triticale seeds differ from wheat seeds in that they have less uniform germination, especially under suboptimal conditions. We have previously demonstrated that priming pre-aged triticale seeds with the nitric oxide donor sodium nitroprusside markedly increases their germination and mitigates the oxidative stress that accompanies the germination process (Kolupaev et al., 2025). To our knowledge, this was the first study to demonstrate the physiological effects of nitric oxide on triticale plants. The impact of nitric oxide donors on the germination of triticale seeds under unfavourable conditions has not yet been investigated.

In connection with the above, this study aimed to investigate the effect of priming triticale seeds with the nitric oxide donor sodium nitroprusside on their germination and seedling growth under conditions of high temperature (35°C) or simulated drought (12% solution of polyethylene glycol with a molecular weight of 6000 Da; hereinafter, PEG 6000). The objectives of this study also included elucidating the effect of NO on carbohydrate metabolism and the accumulation of low-molecular-weight stress metabolites (soluble carbohydrates, proline, phenolic compounds and anthocyanins) as processes important for osmoregulation and defence against oxidative damage.

MATERIALS AND METHODS

Plant material and experimental design

Seeds of the winter triticale (× *Triticosecale* Wittm. ex A. Camus) cultivar ‘Raritet’ (originator: Yuriev Plant Production Institute of the National Academy of Agrarian Sciences of Ukraine) from the 2024 generation were used in the study. All the seeds underwent disinfection with 5% sodium hypochlorite solution for 15 min, followed by thorough washing with sterile distilled water. Then, some seeds were placed in cups with distilled water for 3 h (with a 1:3 seed-to-water ratio – hydropriming). This procedure alone has been shown to increase seed germination by about 10% (Kolupaev et al., 2024b). In this regard, the hydroprimed seeds served as the control. For the sodium nitroprusside treatment variants, the seeds were incubated in 100 μM sodium nitroprusside solution in diffused light at 24°C for 3 h (with a 1:3 seed-to-solution ratio). Additional experimental variants included seed treatment with a mixture of sodium nitroprusside and the NO scavenger methylene blue (100 μM) (Zhang et al., 2006), also for 3 h. The effective concentrations of these compounds for triticale were previously determined by us (Kolupaev et al., 2025). After priming, the seeds from all variants were dried in a thermostat at 24°C and 40% humidity for 24 h.

The treated seeds were divided into three groups based on the germination conditions: (1) germination under optimal conditions (75 grains in each Petri dish on double filters moistened with 8 mL of distilled water at 24°C); (2) germination under heat stress conditions (75 grains in each Petri dish on double filters moistened with 8 mL of distilled water at 35°C); and (3) germination under PEG-simulated drought conditions (75 grains in each Petri dish on double filters moistened with 8 mL of 12% PEG 6000 at 24°C). All variants were germinated in dark thermostats.

Indicators characterising seed germination and seedling growth were evaluated according to the method described by Zhou et al. (2021), with modifications. The germination index (GI) was calculated using the formula Σ(Gt/Dt), where Gt is the per cent of germinated seeds and Dt is the corresponding time to Gt in days. Evaluation was carried out after 1, 2, and 3 days from the beginning of seed germination. After three days, the seed germination (number of germinated grains as a percentage of the total number of seeds) and biomass of the shoots and roots of the seedlings were determined. Using the total biomass of seedlings, the vigour index (VI) was calculated using the formula VI = FW × GI, where FW is the fresh weight of seedlings (mg) and GI is the germination index. Also, after three days, the water content of the shoots was determined by drying them at 103°C to a constant weight.

For all biochemical analyses (except for the determination of amylase activity), only the shoots of three-day-old seedlings were used, as it was impossible to wash the roots free of osmotic residues without damaging them, which could affect the results. Amylase activity was determined in the grains after 24 and 48 h from the start of germination.

Determination of nitrogen oxide content

The nitrogen oxide content in shoots was determined using the method described by Zhou et al. (2005), with some modifications. It is based on converting the NO in plant material to nitrite and determining its concentration in the Griess reaction. A sample of freshly cut plant material was homogenised on ice in a 50 mM acetate buffer (pH 3.6) with the addition of 2% zinc acetate. The homogenate was centrifuged at 2–4°C in an MPW 350R centrifuge (MPW MedInstruments, Poland) at 8000 g for 15 min, then 250 mg of activated charcoal was added to 10 mL of the supernatant. The mixture was filtered through a paper filter, and then 2 mL of the filtrate was mixed with 1 mL of 1% Griess reagent in 12% acetic acid. After 30 min, the absorbance of the solution was determined using a UV-1280 spectrophotometer (Shimadzu, Japan) at 530 nm. Sodium nitrite solutions were used as standards. The NO content was expressed in nmol/g of fresh weight.

Amylase activity analysis

Total amylase activity in seeds was determined on starch agar plates using the ImageJ software (Yastreb et al., 2025). The grains were cut with a sharp lancet. Grain halves without embryos were placed, cut side down, in Petri dishes on plates containing 1% agar and 0.2% starch, and incubated at 24°C in a thermostat for three hours. Then, the gels were poured with 10 mL of diluted Lugol's solution (0.04% I_2 in 0.1% KI) and treated for 5 min. After removing the solution with an automated pipette, the image was captured using a Samsung SM-N9750 camera on a glass covered with paper and illuminated from below. The images were analysed using the ImageJ software (version 1.54g). Colour images were converted to single-channel half-tone images to eliminate variability caused by colour information and analyse differences in colour intensity. Masks of the bright halos surrounding the grains were created on the preprocessed images using the selection tools in ImageJ, excluding areas cut by the grain (Yastreb et al., 2025). The pixel areas of the selected regions were measured using ImageJ software. The results were converted to mm^2 per hour units.

Determination of soluble carbohydrate content

The total soluble carbohydrate content in seedling shoots was determined using a modified Roe (1954) method (Kolupaev et al., 2022a). The plant material was homogenised in distilled water and boiled in a water bath for 10 min. Equal volumes of 30% zinc sulphate and 15% potassium ferrocyanide were then added to precipitate proteins. The samples were stirred and then filtered through paper filters, after which they were diluted with distilled water as necessary. For the assay, 1 mL of the diluted extract was mixed with 3 mL of anthrone reagent. For the comparison solution, 1 mL of purified water was used instead of the extract. The samples were boiled in a water bath for seven minutes, cooled, and their absorbance was determined at 610 nm using a UV-1280 spectrophotometer (Shimadzu, Japan). D-Glucose was used as a standard.

Determination of proline content

Proline content was measured using the method described by Bates et al. (1973) with slight modifications. Shoot samples were homogenised in distilled water, boiled for 10 min, cooled, and filtered through paper filters. In the reaction tubes, 1 mL of the extract, glacial acetic acid, and the ninhydrin reagent were mixed. The tubes were sealed with foil lids and heated in a boiling water bath for 1 h. Absorbance was measured at 520 nm. L-Proline was used as a standard.

Analysis of secondary metabolite content

To determine the total phenolic and anthocyanin content, the shoots were homogenised in 10 mL of 80% ethanol. The mixture was extracted for 20 min. at room temperature and then centrifuged at 8000 g for 15 min. To assay the phenolic compound content, 0.5 mL of the supernatant, 8 mL of distilled water, and 0.5 mL of Folin's reagent were mixed in reaction tubes. The tubes were stirred, and then 1 mL of 10% sodium carbonate was added after 3 min. After one hour, the absorbance of the reaction mixture was measured at 725 nm (Bobo-García et al., 2015). The phenolic compound content was expressed in micromoles of gallic acid per gram of fresh weight.

Before determining the anthocyanin content, the supernatant was acidified with hydrochloric acid to a final concentration of 1% (Nogués & Baker, 2000). Absorbance was measured at a wavelength of 530 nm. The results were expressed as an absorbance index per gram of fresh weight in relative units.

Determination of hydrogen peroxide and malondialdehyde content

To measure the hydrogen peroxide content, the shoot samples were homogenised in a 5% trichloroacetic acid solution on ice. The homogenate was centrifuged at 4°C and 8000 g for 10 min. The amount of H_2O_2 in the supernatant was determined using the ferricyanide method (Sagisaka, 1976). This method is based on the oxidation of Fe^{2+} to Fe^{3+} by hydrogen peroxide in an acidic medium, followed by the formation of a coloured complex of ferric and thiocyanate ions. Hydrogen peroxide solutions were used as standards.

To determine the content of lipid peroxidation products, primarily malondialdehyde, shoot samples were homogenised in either a solution of 0.25% thiobarbituric acid in 10% trichloroacetic acid (experimental sample) or 10% trichloroacetic acid alone (control sample). The mixtures were boiled in foil-covered test tubes in a water bath for 30 minutes. Then, the samples were cooled and centrifuged for 15 min at 10,000 g. Afterwards, the absorbance of the supernatant was measured at 532 nm (main signal) and 600 nm (non-specific light absorbance, which was subtracted from the main result, A_{532}) (Yastreb et al., 2023). The malondialdehyde content was calculated using the molar extinction coefficient ($E = 1.55 \times 10^5$ M^{-1} cm^{-1}).

Replication of experiments and statistical processing of results

When evaluating the effects of stress factors and treatments on the studied compounds on seed germination and seedling biomass, 75 grains or seedlings were used in each replicate, and each experimental variant had at least three replicates. For biochemical assays (except for amylase activity), each sample consisted of at least 12 shoots, and the assays were performed in triplicate. Amylase activity was determined in five replicates, each in a separate Petri dish containing all variants of the experiment with four grains each.

One-factor analysis of variance (ANOVA), followed by Tukey's multiple comparisons test, was used to determine the significance of the differences among the variants of the studied parameters. The figures display the mean values from three biological replicates, along with their standard errors. Different letters indicate significant differences ($p \leq 0.05$). Pearson's correlation coefficients and their significance were calculated in Excel. Principal component analysis (PCA) was performed using R. When a heat map of changes in the studied parameters was constructed, all values were preliminarily normalised from 0 to 1.

RESULTS

Seed germination and seedling growth

At a temperature of 35°C, the germination index decreased by nearly 20% (Fig. 1A). Pretreating the seeds with sodium nitroprusside slightly mitigated this effect, but the difference was not significant at $p \leq 0.05$. The combined treatment of seeds with sodium nitroprusside and the scavenger NO methylene blue did not affect the germination index compared to the index in the variant with high temperature alone.

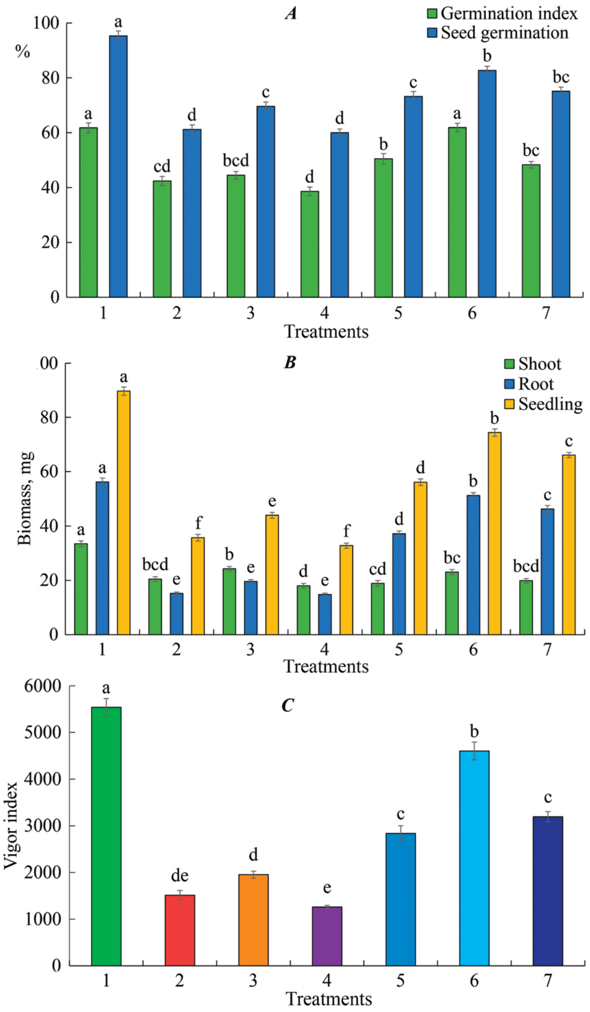


Fig. 1. Germination index and seed germination per cent (A), biomass of seedlings and their organs (B), and vigour index of triticale (C). Treatments: 1 – control; 2 – heat stress; 3 – heat stress + sodium nitroprusside; 4 – heat stress + sodium nitroprusside + methylene blue; 5 – PEG 6000; 6 – PEG 6000 + sodium nitroprusside; 7 – PEG 6000 + sodium nitroprusside + methylene blue. Mean values and their standard errors for three replicates are presented; different letters (a–d) above columns indicate significant differences at $p \leq 0.05$ according to Tukey's LSD test for each indicator (organ).

PEG-simulated drought caused a significant decrease in the germination index at $p \leq 0.05$. However, this decrease was smaller than that caused by heat stress (Fig. 1A). Meanwhile, treating seeds with sodium nitroprusside increased the germination index to the control level. However, the addition of methylene blue to the NO donor solution during seed priming completely negated the positive effect of the nitric oxide donor on the germination index.

The germination rate of triticale seeds in the control group was over 95% but decreased to nearly 60% when the seeds were germinated at a high temperature. Pretreatment with sodium nitroprusside increased seed germination at high temperatures; while, in the presence of the NO scavenger methylene blue, this effect was not evident (Fig. 1A). Seed germination decreased to approximately 73% under the influence of 12% PEG 6000. Treating seeds with the NO donor increased it by almost 10%, but methylene blue reduced this effect.

Shoot biomass was significantly reduced when seeds were germinated at high temperatures (Fig. 1B). The treatment with sodium nitroprusside slightly mitigated this effect, though it was not significant at $p \leq 0.05$. In the variant with a mixture of sodium nitroprusside and methylene blue seed treatment, shoot mass was lower than in the NO donor priming variant. Osmotic stress, as well as heat stress, significantly reduced shoot biomass (Fig. 1B). Sodium nitroprusside priming showed a tendency to increase shoot biomass under PEG-simulated drought conditions. However, this effect was not significant at $p \leq 0.05$. Shoot mass in the variant with priming using a combination of sodium nitroprusside and methylene blue was not significantly different from that in the PEG 6000 treatment variants without priming or with sodium nitroprusside seed priming.

High-temperature seed germination resulted in strong inhibition of root biomass growth (Fig. 1B). Root biomass was only slightly different when seeds were treated with sodium nitroprusside or sodium nitroprusside and methylene blue compared to the high-temperature variant. PEG 6000 treatment reduced root biomass, but to a lesser extent than heat stress did. Treating seeds with sodium nitroprusside significantly increased root biomass in the osmotic stress variant; however, this effect was diminished in the presence of methylene blue (Fig. 1B).

In general, priming seeds with the NO donor caused a relatively small, yet significant ($p \leq 0.05$), increase in total biomass when seedlings grew at a high temperature (Fig. 1B). At the same time, the methylene blue treatment negated the positive effect of sodium nitroprusside on seedling biomass. A similar effect was observed under PEG-simulated drought conditions. NO donor mitigated the inhibition of seedling biomass accumulation when seeds were grown on the PEG 6000 solution, while methylene blue significantly reduced sodium nitroprusside's positive effect.

The conventional integral parameter, the vigour index, which characterises both seed germination and seedling biomass accumulation, decreased by nearly 3.7-fold under high-temperature conditions compared to the control. Treating seeds with sodium nitroprusside showed a tendency to increase it. However, the vigour index was significantly lower in the presence of methylene blue than in the variant with NO donor treatment alone. Under PEG-simulated drought conditions, pretreating seeds with sodium nitroprusside significantly increased the vigour index, and methylene blue eliminated this effect (Fig. 1C).

Water content in shoots

Seed germination at high temperatures decreased shoot water content by about 4%. In this case, pretreatment of the seeds with sodium nitroprusside or the mixture of sodium nitroprusside and methylene blue did not significantly affect shoot water content (Fig. 2).

Under PEG 6000 treatment, the water content in the shoots of the seedlings decreased significantly, by about 9%, compared to the control. Seed treatment with sodium nitroprusside significantly increased shoot water content under PEG-simulated drought conditions. In the presence of the NO scavenger methylene blue, however, the positive effect of nitric oxide donor treatment on water content was significantly reduced (Fig. 2).

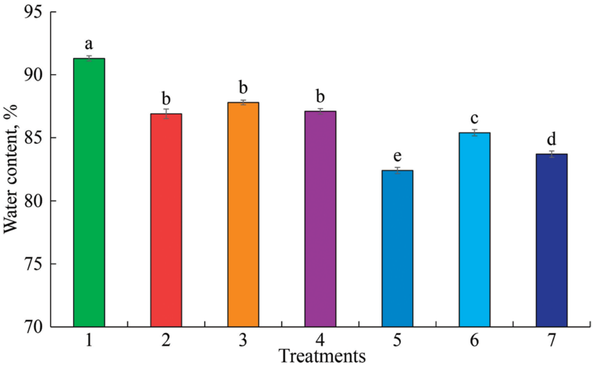


Fig. 2. Water content in triticale seedling shoots. Treatments: 1 – control; 2 – heat stress; 3 – heat stress + sodium nitroprusside; 4 – heat stress + sodium nitroprusside + methylene blue; 5 – PEG 6000; 6 – PEG 6000 + sodium nitroprusside; 7 – PEG 6000 + sodium nitroprusside + methylene blue. Mean values and their standard errors for three replicates are presented; different letters (a–e) above columns indicate significant differences at $p \leq 0.05$ according to Tukey's LSD test.

Nitric oxide content in triticale seedling shoots

High-temperature seed germination increased the nitric oxide content in the shoots of seedlings (Fig. 3). Pretreating seeds with the NO donor sodium nitroprusside increased the amount of endogenous NO in shoots under high temperature conditions, while methylene blue eliminated this effect.

PEG 6000 treatment tended to increase the NO content in shoots slightly, but this effect was not significant at $p \leq 0.05$ (Fig. 3). Sodium nitroprusside seed priming increased the endogenous NO content in shoots; however, in the presence of the NO scavenger methylene blue, this effect was not evident.

Total amylase activity in triticale grains

After 24 hours of germination at a high temperature, the amylase activity in grains did not differ much from the corresponding value in the control (Fig. 4). Treatment with the nitric oxide donor sodium nitroprusside caused a significant increase in amylase activity in the grains. This effect was partially reduced in the presence of the NO scavenger methylene blue.

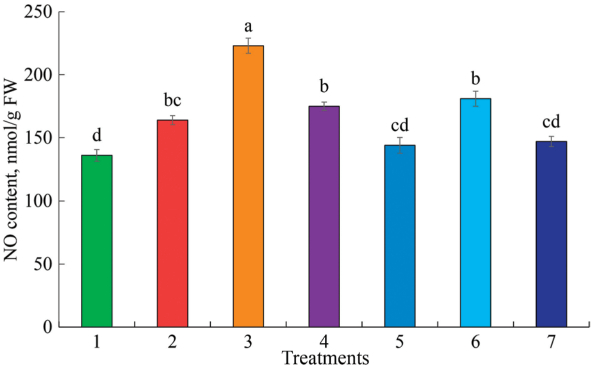


Fig. 3. Nitric oxide (NO) content in triticale seedling shoots. Treatments: 1 – control; 2 – heat stress; 3 – heat stress + sodium nitroprusside; 4 – heat stress + sodium nitroprusside + methylene blue; 5 – PEG 6000; 6 – PEG 6000 + sodium nitroprusside; 7 – PEG 6000 + sodium nitroprusside + methylene blue. Mean values and their standard errors for three replicates are presented; different letters (a–d) above columns indicate significant differences at $p \leq 0.05$ according to Tukey's LSD test. See the Materials and Methods section for more information.

After 48 hours from the start of germination, amylase activity in the control increased significantly (almost twofold). However, under heat stress conditions, the increase in amylase activity on the second day of seed germination was less significant than in the control group. Meanwhile, priming grains with sodium nitroprusside resulted in a significant increase in enzyme activity after 48 hours of germination at 35°C. However, treatment with methylene blue completely eliminated this effect of the NO donor (Fig. 4).

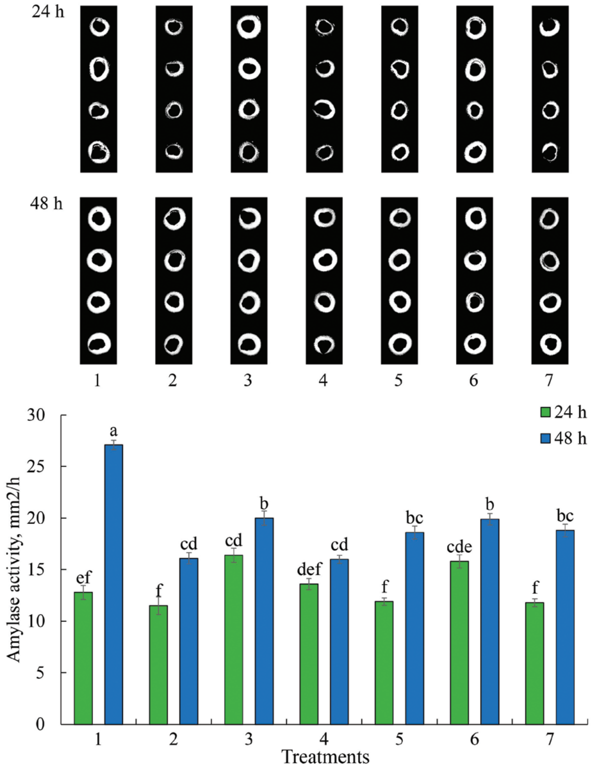


Fig. 4. Total amylase activity in triticale grains 24 and 48 hours after germination. Treatments: 1 – control; 2 – heat stress; 3 – heat stress + sodium nitroprusside; 4 – heat stress + sodium nitroprusside + methylene blue; 5 – PEG 6000; 6 – PEG 6000 + sodium nitroprusside; 7 – PEG 6000 + sodium nitroprusside + methylene blue. Mean values and their standard errors for five replicates are presented; different letters (a–e) above columns indicate significant differences at $p \leq 0.05$ according to Tukey's LSD test. See the Materials and Methods section for more information.

In the PEG 6000 treatment variant, amylase activity 24 hours after the start of grain germination did not differ significantly from the control (Fig. 4). Seed pretreatment with the NO donor increased enzyme activity in grains during the first day of germination in the presence of PEG 6000. However, this effect was not apparent in the presence of the NO scavenger methylene blue. On the second day of seed germination under PEG-simulated drought conditions, amylase activity increased while the effects of priming the seeds with sodium nitroprusside and methylene blue levelled off (Fig. 4).

Content of compatible osmolytes

Sugars and proline are the main organic osmolytes in plant cells. When seeds were germinated at a high temperature, the total soluble carbohydrate content in the shoots of the resulting seedlings decreased (Fig. 5A). Treating seeds with the nitric oxide donor sodium nitroprusside completely eliminated the negative effect of high-temperature stress on sugar content in shoots. Meanwhile, the nitric oxide scavenger methylene blue negated the effect of the NO donor.

When seeds were germinated under PEG-induced drought stress, the sugar content in the shoots of the seedlings was significantly lower than in the control

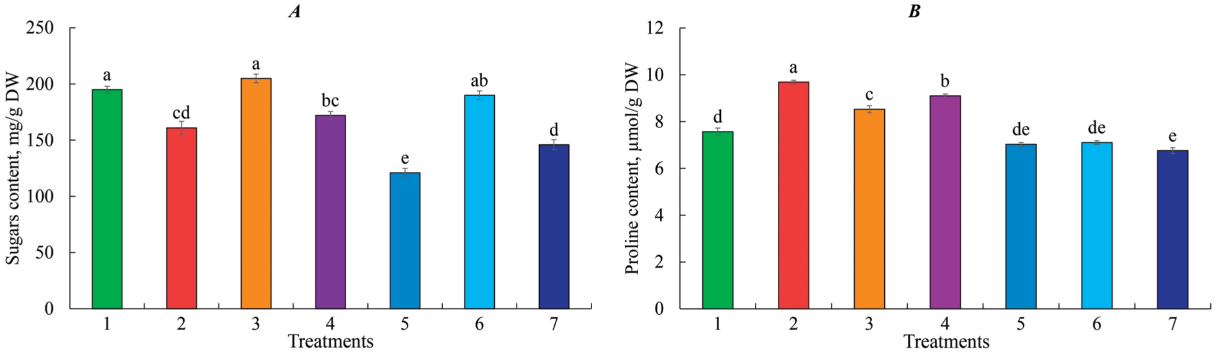


Fig. 5. Sugar (A) and proline (B) content per dry weight (DW) in shoots of triticale seedlings. Treatments: 1 – control; 2 – heat stress; 3 – heat stress + sodium nitroprusside; 4 – heat stress + sodium nitroprusside + methylene blue; 5 – PEG 6000; 6 – PEG 6000 + sodium nitroprusside; 7 – PEG 6000 + sodium nitroprusside + methylene blue. Mean values and their standard errors for three replicates are presented; different letters (a–e) above columns indicate significant differences at $p \leq 0.05$ according to Tukey's LSD test. See the Materials and Methods section for more information.

group (Fig. 5A). However, treating seeds with the NO donor increased the sugar content in the shoots under drought stress to the same level as the control. This effect was negated, mainly in the presence of methylene blue.

High temperature during the germination of triticale seeds increased the proline content in the shoots of seedlings (Fig. 5B). This effect was weaker in seedlings grown from seeds primed with sodium nitroprusside. In the presence of the NO scavenger methylene blue, the inhibitory effect of the nitric oxide donor on proline accumulation in shoots was diminished.

Under PEG 6000 treatment conditions, the proline content in the shoots of triticale seedlings did not differ from the control (Fig. 5B). Meanwhile, priming seeds with NO donor sodium nitroprusside or the mixture of NO donor and its scavenger methylene blue did not affect proline content in shoots under PEG-induced drought stress.

Content of secondary metabolites

Phenolic and polyphenolic (flavonoid) compounds are multifunctional plant stress metabolites that have antioxidant, membrane-protective, and partial osmoprotective properties (Shomali et al., 2022; Qaderi et al., 2023). Seed germination at high temperatures resulted in decreased total phenolic compound levels in shoots. Pretreatment with sodium nitroprusside or its mixture with methylene blue did not affect the temperature-induced decrease in total phenolic compounds (Fig. 6A).

In contrast to high-temperature stress, seed germination under PEG 6000 treatment conditions caused a slight increase in the total content of phenolic compounds in shoots. Some fluctuations in the amount of phenolic compounds were observed in the shoots of seedlings obtained from seeds treated with the nitric oxide donor or the mixture of the donor and NO scavenger. However, these effects were not significant at $p \leq 0.05$ (Fig. 6A).

A substantial decrease in anthocyanin content in shoots was observed when seeds were germinated under high-temperature conditions (Fig. 6B). Pretreating the seeds with the NO donor sodium nitroprusside or its mixture with the nitric oxide scavenger methylene blue did not affect the manifestation of this effect.

Similar to high temperatures, a decrease in the anthocyanin content of seedling shoots was observed under PEG-induced drought conditions (Fig. 6B). However, pretreatment with the nitric oxide donor almost completely alleviated the negative effects of PEG 6000 treatment on anthocyanin content. At the same time, this effect of the NO donor was not manifested in the presence of its scavenger, methylene blue.

Contents of oxidative stress markers

Heat stress during the germination of triticale seeds significantly increased the peroxide content of the shoots (Fig. 7A). Priming the seeds with sodium nitroprusside completely prevented the heat-induced increase in H_2O_2 in the shoots. In the presence of the NO scavenger methylene blue, the effect of the nitric oxide donor on the amount of hydrogen peroxide was much weaker.

When seeds were germinated under PEG-simu-

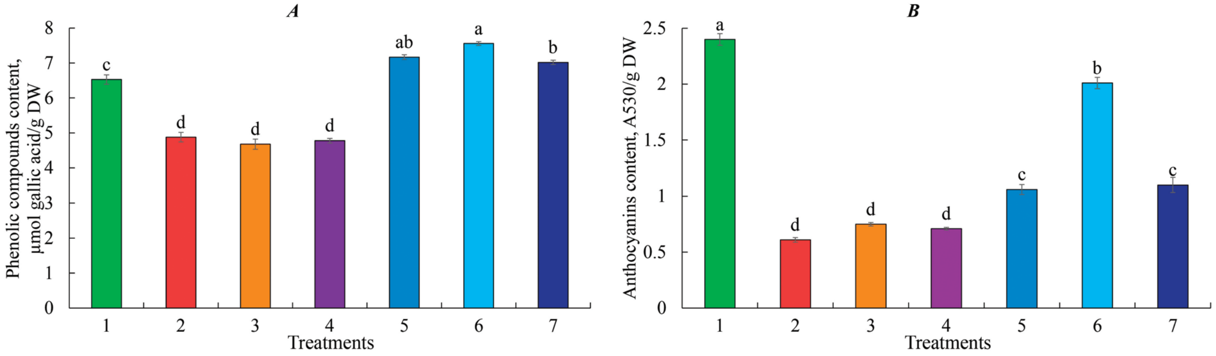


Fig. 6. Content of phenolic compounds (A) and anthocyanins (B) per dry weight (DW) in shoots of triticale seedlings. Treatments: 1 – control; 2 – heat stress; 3 – heat stress + sodium nitroprusside; 4 – heat stress + sodium nitroprusside + methylene blue; 5 – PEG 6000; 6 – PEG 6000 + sodium nitroprusside; 7 – PEG 6000 + sodium nitroprusside + methylene blue. Mean values and their standard errors for three replicates are presented; different letters (a–d) above columns indicate significant differences at $p \leq 0.05$ according to Tukey's LSD test. See the Materials and Methods section for more information.

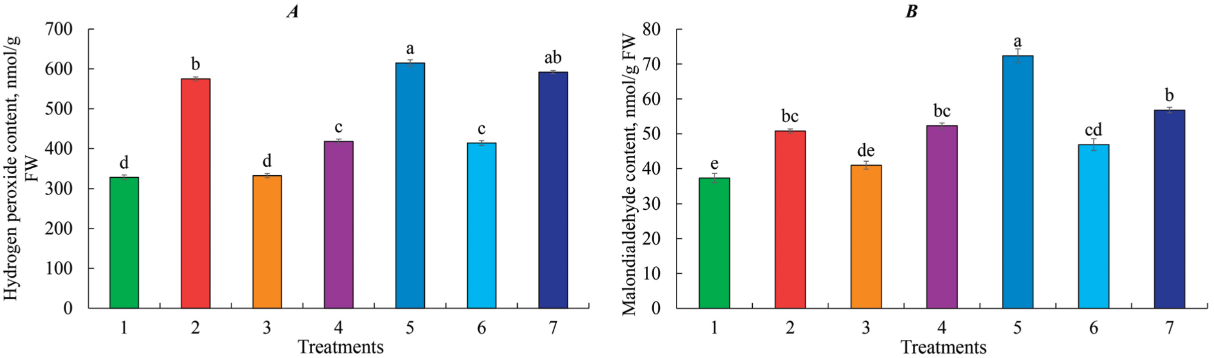


Fig. 7. Hydrogen peroxide (A) and malondialdehyde (B) content per fresh weight (FW) in shoots of triticale seedlings. Treatments: 1 – control; 2 – heat stress; 3 – heat stress + sodium nitroprusside; 4 – heat stress + sodium nitroprusside + methylene blue; 5 – PEG 6000; 6 – PEG 6000 + sodium nitroprusside; 7 – PEG 6000 + sodium nitroprusside + methylene blue. Mean values and their standard errors for three replicates are presented; different letters (a–d) above columns indicate significant differences at $p \leq 0.05$ according to Tukey's LSD test. See the Materials and Methods section for more information.

lated drought conditions, the amount of H_2O_2 in the shoots was twice as high as in the control group (see Fig. 7A). Meanwhile, priming seeds with the NO donor negated the effect of drought on hydrogen peroxide content in shoots. Conversely, treatment with a mixture of sodium nitroprusside and the nitrogen oxide scavenger methylene blue resulted in H_2O_2 content in shoots similar to that observed under PEG 6000 treatment.

Seed germination at high temperatures resulted in increased levels of an important oxidative stress marker, malondialdehyde (a lipid peroxidation product), in the shoots (Fig. 7B). Priming seeds with the nitric oxide donor significantly mitigated this effect. Conversely, in the presence of the nitric oxide scavenger methylene blue, sodium nitroprusside treatment did not prevent the development of high-temperature-induced oxidative stress.

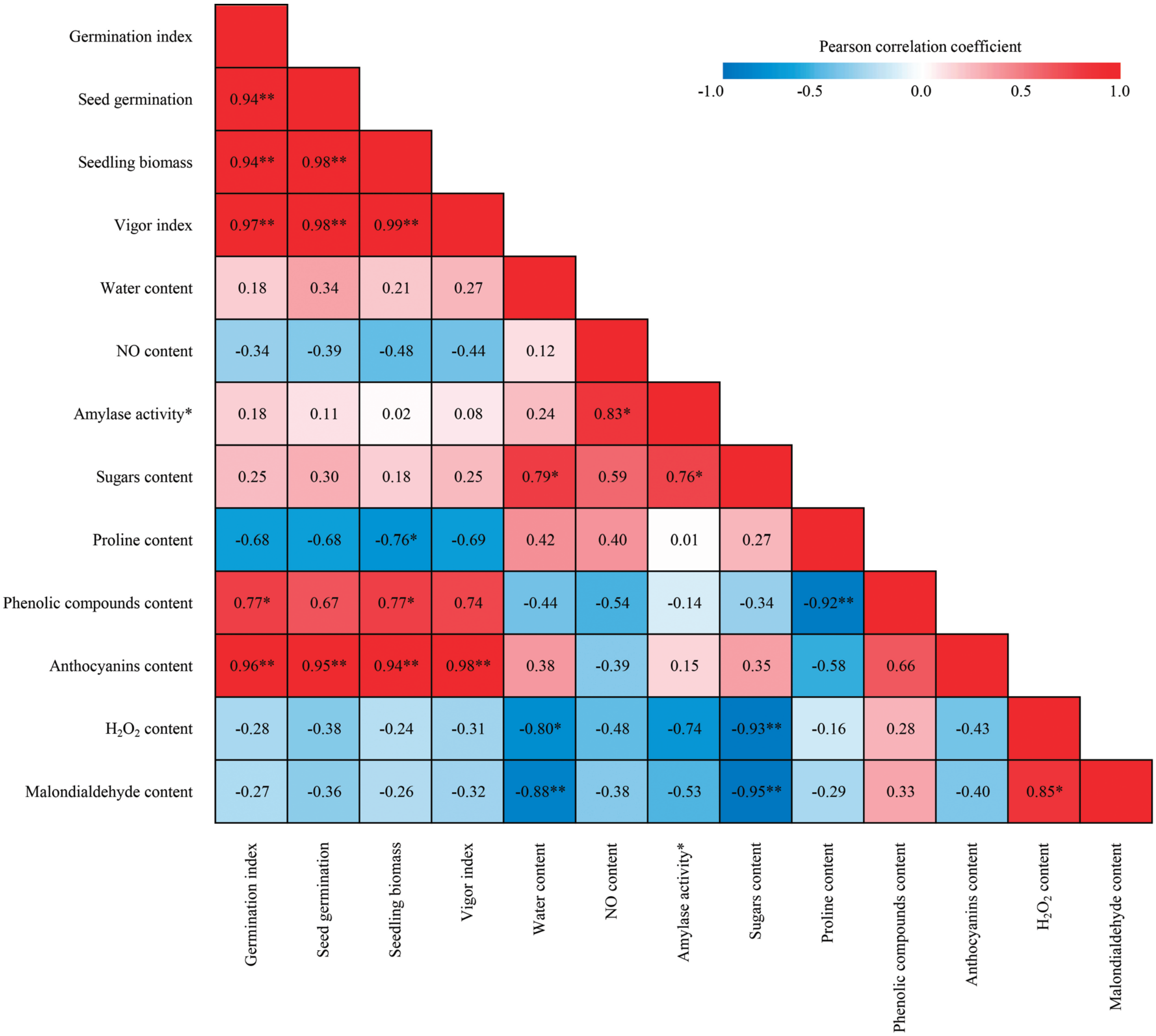
Under PEG 6000 treatment conditions, the accumulation of malondialdehyde in shoots was more significant than under high temperature conditions (Fig. 7B). In this case, sodium nitroprusside priming prevented the accumulation of the oxidative stress marker in the shoots. Meanwhile, the protective effect of the NO donor was significantly reduced in the presence of the NO scavenger.

Correlations between physiological and biochemical parameters

As expected, very high correlation values were observed between various indices characterising seed germination and seedling growth, such as germination index, seed germination, seedling biomass, and vigour index. This indicates a close relationship between the processes of seed germination and seedling growth (Fig. 8). The content of anthocyanins showed a very close direct correlation with all of these growth indices. Total phenolic compound content was also closely correlated with the germination index and seedling biomass. Meanwhile, proline content showed a high inverse correlation with indices characterising seed germination and seedling growth. For the seedling biomass index, this correlation was significant at $p \leq 0.05$. Additionally, attention should be paid to the high direct correlation between amylase activity in grains and sugar content in shoots, as well as tissue water content. Conversely, the amount of oxidative stress markers (hydrogen peroxide and malondialdehyde) was closely inversely correlated with tissue water content. Their content was also in a close negative correlation with sugar content, which may indicate the role of sugars in the antioxidant defence system.

Principal component analysis

The results of the principal component analysis (PCA) indicate that the first principal component (PC1) explains 52.8% of the variance. In comparison, the second principal component (PC2) explains 33.8% (Fig. 9). Therefore, PC1 accounts for more variability in the dataset than PC2. The control variant, which involved PEG 6000 treatment combined with sodium nitroprusside treatment, and the variant



	Germination index	Seed germination	Seedling biomass	Vigor index	Water content	NO content	Amylase activity*	Sugars content	Proline content	Phenolic compounds content	Anthocyanins content	H_2O_2 content	Malondialdehyde content
Germination index													
Seed germination	0.94**												
Seedling biomass	0.94**	0.98**											
Vigor index	0.97**	0.98**	0.99**										
Water content	0.18	0.34	0.21	0.27									
NO content	-0.34	-0.39	-0.48	-0.44	0.12								
Amylase activity*	0.18	0.11	0.02	0.08	0.24	0.83*							
Sugars content	0.25	0.30	0.18	0.25	0.79*	0.59	0.76*						
Proline content	-0.68	-0.68	-0.76*	-0.69	0.42	0.40	0.01	0.27					
Phenolic compounds content	0.77*	0.67	0.77*	0.74	-0.44	-0.54	-0.14	-0.34	-0.92**				
Anthocyanins content	0.96**	0.95**	0.94**	0.98**	0.38	-0.39	0.15	0.35	-0.58	0.66			
H_2O_2 content	-0.28	-0.38	-0.24	-0.31	-0.80*	-0.48	-0.74	-0.93**	-0.16	0.28	-0.43		
Malondialdehyde content	-0.27	-0.36	-0.26	-0.32	-0.88**	-0.38	-0.53	-0.95**	-0.29	0.33	-0.40	0.85*	

Fig. 8. Pearson correlation between growth indices and biochemical parameters during triticale seed germination. *Correlation is significant at $p \leq 0.05$. **Correlation is significant at $p \leq 0.01$. See the text for other explanations.

with PEG 6000 treatment combined with seed priming using a mixture of the NO donor sodium nitroprusside and the NO scavenger methylene blue, were located in the positive region of PC1. However, the latter is nearly on the PC2 axis, indicating a much weaker association with PC1. Variables characterising seed germination, such as germination index, biomass of whole seedlings and their organs, and vigour index, as well as anthocyanin content, carry a strongly pronounced positive load on PC1. Indices of amylase activity, sugars, phenolic compounds, and water content in seedlings are also in the positive part of PC1; however, their relationship with PC1 is less pronounced.

In the negative part of PC1 were variants with heat stress, its combination with seed treatment using sodium nitroprusside and methylene blue, as well as variants with osmotic stress in combination with seed priming with a mixture of sodium nitroprusside and methylene blue and heat stress in combination with sodium nitroprusside treatment alone. However, the latter two variants are located near the PC2 axis, indicating that they are less associated with PC1. Variables that characterise the intensity of oxidative stress (content of H_2O_2 and malondialdehyde in shoots of seedlings), as well as the content of proline and nitric oxide, bear a negative load on PC1.

As for PC2, most variants of the experiment,

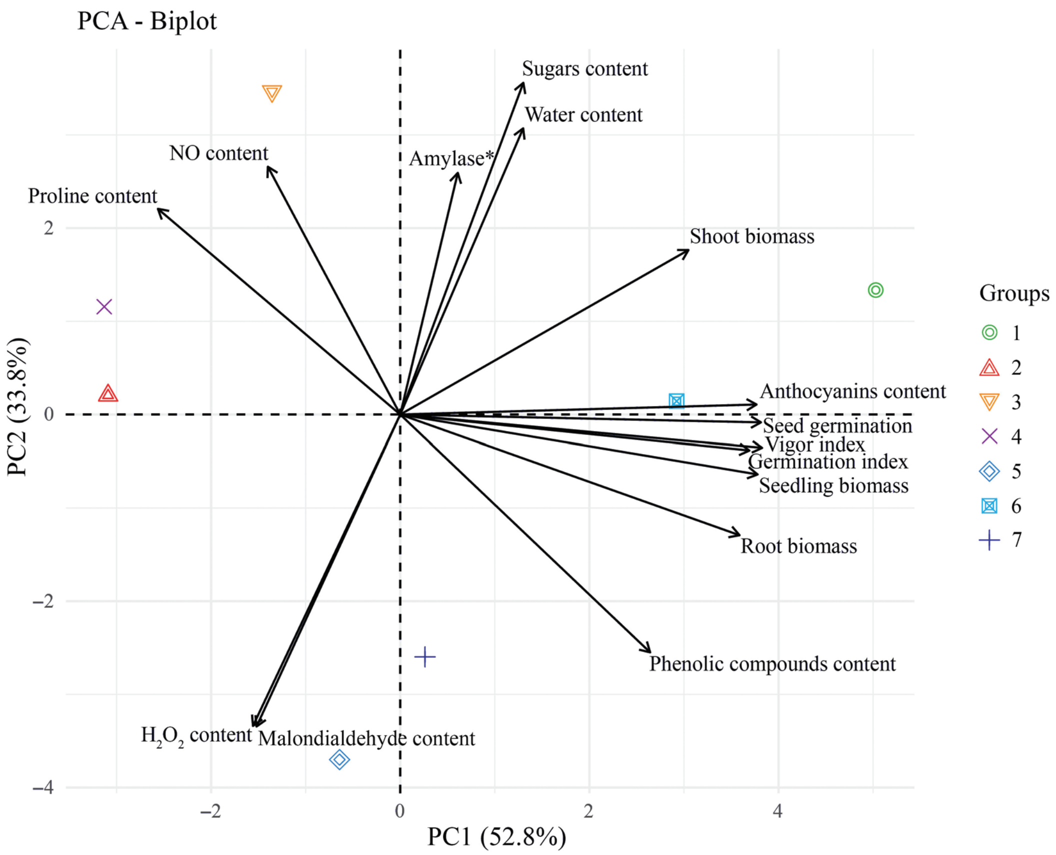


Fig. 9. Principal component analysis of the effect of heat stress, osmotic stress, nitric oxide donor sodium nitroprusside, nitric oxide scavenger methylene blue, and a combination of the factors on germinating triticale grains. Treatments: 1 – control; 2 – heat stress; 3 – heat stress + sodium nitroprusside; 4 – heat stress + sodium nitroprusside + methylene blue; 5 – PEG 6000; 6 – PEG 6000 + sodium nitroprusside; 7 – osmotic stress + sodium nitroprusside + methylene blue. *For calculations, the values of amylase activity observed 24 hours after the start of grain germination were used. See the text for more information.

including the control, fall within its positive range. Only variants involving PEG 6000 treatment or a combination of seed treatment with a mixture of sodium nitroprusside and methylene blue fall within its negative range. Significant positive load on PC2 is carried by indicators such as amylase activity in grains and the content of sugars, water, nitric oxide, and proline in shoots. Conversely, the negative load on PC2 is primarily influenced by markers of oxidative stress and, to a lesser extent, by the content of phenolic compounds and root biomass.

The most notable relationships of nitric oxide donor effects with certain biochemical indices should be emphasised. Thus, the vector of anthocyanins content reveals a significant association with the impact of NO donor sodium nitroprusside under PEG-induced but not heat stress (Fig. 9). The vectors of amylase activity and sugars content as well as the content of endogenous nitric oxide indicate an essential role of these parameters in the variant with the effect of sodium nitroprusside under heat stress and a less close relationship with the effect of NO donor under the action of PEG 6000 treatment. Vectors reflecting oxidative stress indicators, namely hydrogen peroxide and malondialdehyde content, are associated with variants that experience high temperatures and, especially, drought, as indicated on the left side of the graph. It should be noted that the groups of variants with the action of the NO donor together with the NO scavenger methylene blue on the background of PEG 6000 treatment or high temperature are located close to the corresponding groups with the action of stress factors themselves, indicating the elimination of the protective effects of the NO donor sodium nitroprusside (Fig. 9).

DISCUSSION

This study is the first to report the positive effect of sodium nitroprusside on triticale seed germination under heat and osmotic stress. In general, a considerable amount of data has been accumulated in the literature on the positive effects of exogenous nitric oxide on the germination of seeds of different plant species (Bethke et al., 2007; Krasuska et al., 2015; Maslennikova et al., 2025). These effects have been observed during seed germination under both normal and stressful conditions. As previously noted, we have demonstrated that sodium nitroprusside priming enhances the germination of aged triticale seeds under optimal conditions (Kolupaev et al., 2025). Data have been obtained on the positive effect of NO donors on seed germination under temperature and simulated drought stress conditions for other cereal species, particularly wheat (Zhang et al., 2003; Duan et al., 2007; Bibi et al., 2020). Wheat seedlings also show less inhibition of organ growth under salt stress in the presence of sodium nitroprusside (Maslennikova et al., 2023). Additionally, the positive effects of the natural NO donor L-arginine on wheat seedling survival after heat stress have been demonstrated (Karpets et al., 2018).

The positive effects of sodium nitroprusside seed priming on most germination and growth indicators were reduced or eliminated in the presence of the nitric oxide scavenger methylene blue (Fig. 1, 10). This indicates that sodium nitroprusside acted specifically as a nitric oxide donor under the experimental conditions. Previous studies have shown that the positive effects of sodium nitroprusside priming on the germination of aged triticale seeds are not observed in the presence of methylene blue (Kolupaev et al., 2025). Zhang et al. (2006) have demonstrated that methylene blue negates the beneficial effects of sodium nitroprusside on corn seedling growth under salt stress conditions. Additionally, methylene blue is found to eliminate the enhancement of tomato seed germination caused by sodium nitroprusside treatment under low-temperature conditions (Amooaghaie & Nikzad, 2013). Therefore, it is reasonable to conclude that

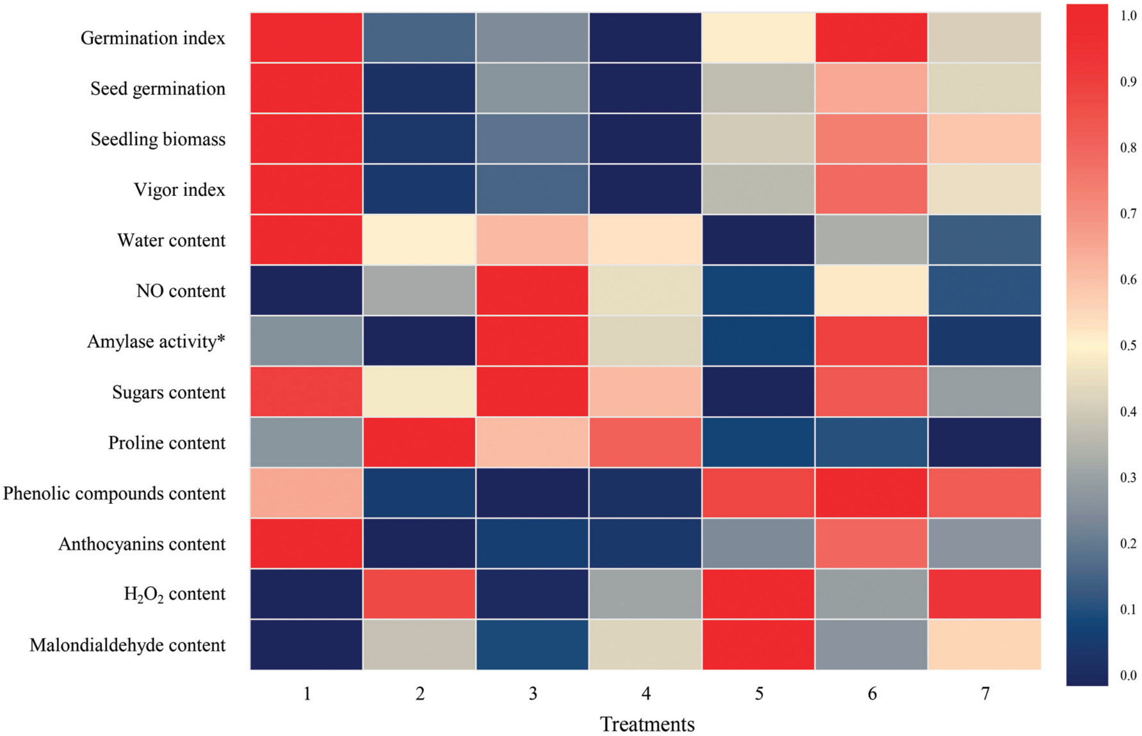


Fig. 10. Heat map showing changes in growth and biochemical parameters during the germination of triticale seeds. All parameters were normalised to the range of 0 to 1 before constructing the heat map. Treatments: 1 – control; 2 – heat stress; 3 – heat stress + sodium nitroprusside; 4 – heat stress + sodium nitroprusside + methylene blue; 5 – PEG 6000; 6 – PEG 6000 + sodium nitroprusside; 7 – PEG 6000 + sodium nitroprusside + methylene blue. *For calculations, the values of amylase activity observed 24 hours after the start of grain germination were used. See the text for other explanations.

the positive effects of sodium nitroprusside on seed germination, including under adverse conditions, are due to its action as a nitric oxide donor rather than the potential side effects of other sodium nitroprusside decomposition products such as cyanides (Mur et al., 2013).

It is noteworthy that under the conditions of our experiments, sodium nitroprusside treatment caused an increase in the content of endogenous nitric oxide in shoots (Fig. 3). This provides direct evidence that the effects of sodium nitroprusside seed priming are mediated by NO. However, the increase in endogenous NO observed in the experiment may not be associated with the direct accumulation of NO released during sodium nitroprusside decomposition in tissues, but rather with the stimulation of endogenous NO synthesis by the treatment. The effects of sodium nitroprusside treatment on the induction of endogenous NO synthesis have been demonstrated in peach fruits, for example. Soaking the fruits in sodium nitroprusside solutions increased their NO content over the course of two weeks of subsequent storage at low temperatures. This effect was accompanied by increased activity and expression of the nitrate reductase gene as well as increased nitric oxide synthase (NOS)-like activities. These are the main enzymatic systems that synthesise NO (Tian et al., 2020). A similar mechanism may be responsible for the increase in NO content in the shoots of three-day-old triticale seedlings obtained from sodium nitroprusside-primed seeds. It appears less probable that NO is released from sodium nitroprusside that has permeated the grains and potentially the sprouts, considering this nitric oxide donor is not stable (Floryszak-Wieczorek et al., 2006). In any case, the increase in endogenous NO in shoots is associated with sodium nitroprusside's primary action as a nitric oxide donor since the increase was eliminated by the NO scavenger methylene blue (Fig. 3). Notably, stress effects themselves caused an increase in nitric oxide content in shoots; the effect of heat stress was significant at $p \leq 0.05$ and the effect of osmotic stress manifested at the trend level. Several studies have demonstrated the necessity of increasing endogenous NO content to develop plant resistance to abiotic stresses (Yemets et al., 2019). In our experiments, an increase in nitric oxide content in the shoots of seedlings under heat and simulated drought stress appeared to contribute to adaptive responses to these stressors. This assumption is supported by the observed biochemical changes (increased sugar and anthocyanin content in shoots and decreased oxidative stress markers under stress conditions) when treated with a nitric oxide donor.

Importantly, treating triticale grains with sodium nitroprusside increased amylase activity, which was particularly significant after 24 hours (Fig. 4). Similar increases in total amylase activity, as well as in the α- and β-forms separately, have been recorded in wheat grains under osmotic and salt stress conditions after treatment with sodium nitroprusside (Zhang et al., 2003; Zheng et al., 2009). The mechanisms by which nitric oxide affects amylase activity are not well understood, though data indicate the possibility of post-translational modifications of β-amylase under the influence of NO (Zhang et al., 2005). Several studies have linked increased amylase activity to an increased germination index and increased biomass of wheat seedling shoots and roots when seeds are germinated under stressful conditions (Zhang et al., 2003; Zheng et al., 2009). Notably, amylase activation by exogenous NO is accompanied by increased sugar content and decreased starch content in germinating wheat grains (Zheng et al., 2009). In our experiments, sodium nitroprusside priming prevented the decrease in sugar content in triticale shoots caused by heat and PEG 6000 treatment (Figs 5 and 10). Meanwhile, vectors for amylase activity in grains, sugar content, and water content in shoots were clustered closely together on the biplot graph (Fig. 9), indicating a close relationship between these indicators. Correlation analysis results also indicate statistically significant relationships between amylase activity and sugar content, as well as between sugar and water content in shoots (Fig. 8). Therefore, it is reasonable to conclude that maintaining elevated sugar content in shoots under conditions of heat and PEG 6000 treatment stress is crucial for osmoregulation and maintaining tissue hydration. The positive effects of sodium nitroprusside on amylase activity, sugar content and tissue hydration in seedlings under stress conditions appear to be associated with nitric oxide formation, as this effect was almost absent in the presence of the NO scavenger, methylene blue (Figs 2, 4 and 5A).

Proline, along with soluble carbohydrates, is also considered an important osmolyte necessary for adaptation to stresses accompanied by tissue dehydration (Mansour & Salama, 2020; Raza et al., 2023). However, we observed only a relatively small increase in proline content in triticale seedling shoots under heat stress (Fig. 5B). Priming the seeds with the NO donor eliminated this heat stress effect. Under heat stress, the proline content was higher in the variant with a combination of sodium nitroprusside and methylene blue seed treatment than in the variant with sodium nitroprusside treatment alone. On the other hand, the proline content remained unchanged under PEG 6000 treatment and was unaffected by sodium nitroprusside or methylene blue. No significant correlation was found between proline content and seedling tissue water content (Fig. 8).

Proline is traditionally considered one of the main osmolytes capable of exerting multifunctional actions associated with osmoprotective, membrane-protective, chaperone, and antioxidant effects (Szabados & Savouré, 2010; Forlani et al., 2019; Ghosh et al., 2022; Raza et al., 2023). However, proline's contribution to stress-protective systems may vary depending on the plant species characteristics. A comparative study of the response of triticale and other cereal seedlings (*Secale cereale* L., *Triticum aestivum* L., and *Triticum durum* L.) to high temperatures has shown that triticale seedlings have the lowest basal proline content and accumulate significantly less proline under stress conditions than other species (Kolupaev et al., 2022b). Therefore, it is likely that proline contributes less to the functioning of stress-protective systems in triticale than in other species. The absence of a pronounced effect of the NO donor on proline content may also be associated with triticale's species characteristics (Fig. 5). However, many studies on different plant species have shown an increase in proline content under normal and stressful conditions when treated with nitric oxide donors. Such effects have been reported in particular in *Triticum aestivum* (Ruan et al., 2004), *Cucumis sativus* L. (Fan et al., 2012), and *Glycine max* L. (Rezayian et al., 2023). The cited works also report increased activity and gene expression of enzymes involved in proline synthesis. Furthermore, it has recently been demonstrated that proline accumulation induced by exogenous NO is associated with the S-nitrosylation of Δ^1-pyrroline-5-carboxylate reductase at Cys-5 (Liu et al., 2024). However, in some plant species, such as *Brassica napus* L. (Hasanuzzaman et al., 2017), *Arabidopsis thaliana* (Yastreb et al., 2017), and *Prunus armeniaca* L. (Bakır et al., 2022), proline content decreases with NO donor treatment, especially under stressful conditions. Differences in species' strategies for adaptation to osmotic stress may also cause differences in proline content changes in response to exogenous nitric oxide treatment. The activation of other adaptive responses by nitric oxide possibly mitigates the stress-induced effect of increased proline content.

In addition to primary stress metabolites, such as sugars and proline, secondary metabolites – which are compounds of a phenolic or polyphenolic (flavonoid) nature – are essential for a plant's adaptation to abiotic stress (Shomali et al., 2022; Qaderi et al., 2023). Their most important effect is antioxidant. In triticale seedlings, an increase in total phenolic compound content was observed under PEG 6000 treatment. In contrast, a decrease was observed under heat stress (Fig. 6). These differences may be due to the stronger growth-inhibiting effect of heat stress (Fig. 1B), which resulted in a more significant expenditure of metabolites during the adaptation process. It is possible that part of the pool of phenolic compounds was used to synthesise lignin, which plays a role in adaptation to osmotic stress (Maslennikova et al., 2023a). Notably, the content of phenolic compounds in triticale seedlings did not change significantly under the action of the nitrogen oxide donor (Fig. 6A). Previously, we observed an increase in phenolic compound synthesis in wheat seedlings, but not in triticale seedlings, when using sodium nitroprusside priming on aged wheat and triticale seeds (Kolupaev et al., 2025). Thus, the effect of nitrogen oxide on the accumulation of secondary metabolites appears to depend on plant species characteristics. Triticale seedlings, which have a relatively low total content of phenolic compounds, accumulate large amounts of anthocyanins under optimal conditions (Fig. 6B). However, under stressful conditions, the anthocyanin pool decreases sharply, apparently due to intense oxidation (Neill & Gould, 2003). Meanwhile, treating seeds with the nitric oxide donor prevented the depletion of the anthocyanin pool under PEG 6000 treatment but was less effective under heat stress. The results of the principal component analysis indicate a significant relationship between the effects of exogenous nitric oxide and the anthocyanin content in triticale seedlings, particularly under PEG 6000 treatment (Fig. 9). This is consistent with the complete elimination of sodium nitroprusside's effect on anthocyanin content under PEG 6000 treatment stress when treated with the NO scavenger methylene blue (Fig. 6B).

A striking manifestation of the protective effect of nitric oxide donors against oxidative damage is evident in the change in hydrogen peroxide and malondialdehyde content in seedling shoots (Fig. 7). Priming seeds with sodium nitroprusside almost eliminated the increase in these oxidative stress indicators caused by heat and PEG 6000 treatment stress. Conversely, treatment with the NO scavenger methylene blue significantly negated the effects of the nitric oxide donor. These results align with literature on the mitigation of oxidative damage by exogenous nitric oxide during osmotic stress (Tian & Lei, 2006; Majeed et al., 2018). The significant contribution of oxidative damage to the inhibition of seed germination and seedling growth is indicated by the location of the hydrogen peroxide content vectors in the negative part of PC1, which are clearly opposite the vectors of seed germination and seedling growth indicators (Fig. 9).

When discussing the results, one should emphasise that the method of using a nitric oxide donor to promote triticale seedling growth under heat and PEG-simulated drought stress involved soaking the seeds in the sodium nitroprusside solution for a short time and then drying them for 24 hours. In this regard, we can discuss the relatively prolonged physiological effects of the NO donor; at the same time, it is likely that these effects are associated with increased endogenous NO formation. This is indicated by the increased NO content found in 3-day-old seedlings in the sodium nitroprusside-treated variants (Fig. 3). The literature also reports more distant effects of sodium nitroprusside plant priming, such as increased endogenous NO content 30 days after pre-sowing seed treatment (Maslennikova et al., 2023b). Additionally, a positive effect of sodium nitroprusside wheat seed priming on antioxidant enzyme activity and sugar content in flag leaves of 98-day-old drought-stressed plants has been reported (Hameed et al., 2021). The same study demonstrated an increase in yield for plants grown from seeds primed with sodium nitroprusside. Based on these results, the authors conclude that "sodium nitroprusside-mediated priming memory" is manifested. However, the molecular mechanisms of the prolonged stress-protective effect of seed priming by nitric oxide donors remain unclear. Hypotheses include the involvement of epigenetic mechanisms and/or the initiation of long-term physiological programmes associated with significant changes in the hormonal status of plants under priming influence (Jatana et al., 2024). Discussion of this material is beyond the scope of our study. In general, there is reason to believe that nitric oxide donors can be an effective tool for increasing plant resistance to abiotic stresses, particularly high temperatures and drought, when used in seed priming technologies. However, the practical use of nitric oxide donors as priming agents should be preceded by research into the characteristics of the corresponding physiological effects in specific species.

CONCLUSION

This study is the first to demonstrate that priming with nitric oxide donors enhances the germination of triticale seeds and the growth of triticale seedlings under conditions of heat stress and simulated drought. The nitric oxide donor effects include increased amylase activity in grains and increased accumulation of soluble carbohydrates in shoots. This contributes to reduced tissue dehydration under stressful conditions. Another pronounced stress-protective reaction stimulated by nitric oxide under drought conditions is maintaining a pool of anthocyanins with high antioxidant activity in seedlings. Priming triticale seeds with sodium nitroprusside also positively affects the antioxidant system of seedlings by eliminating hydrogen peroxide and malondialdehyde accumulation under heat and drought stress conditions.

ACKNOWLEDGEMENTS

This work is part of the project "Comprehensive scientific study of mechanisms of resistance of crop plants to biotic, abiotic, and anthropogenic stress, use of genetic diversity, and creation of stress-resistant cultivars and hybrids", funded by the Ministry of Education and Science of Ukraine (registration number 0125U003530).

Author contributions. IVS – investigations, methodology and data curation; TOY – investigations, methodology, writing and editing; DAT – investigation and data curation; AIO – investigations; MAS – methodology and data curation; NIR – investigations; YEK – investigations, writing, and project administration. All authors have read and agreed to the final version of the manuscript.

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