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PRIMING WITH NO DONOR SODIUM NITROPRUSSIDE TO ACTIVATE GERMINATION AND REDUCE OXIDATIVE DAMAGE IN AGED WHEAT AND TRITICALE SEEDS

SUMMARY

Despite the existence of numerous experimental data on the stimulation of seed germination of various plant species under unfavourable conditions by nitric oxide donors, there are very few data on the effect of exogenous NO on the germination of cereal seeds that have undergone accelerated ageing. In particular, they are lacking for aged seeds of one of the most widespread bread cereals, *Triticum aestivum* L. Any physiological effect of NO on the wheat-rye hybrid triticale (×*Triticosecale*) has not been studied. The aim of this work was to study the effect of priming aged wheat seeds of cultivar Scorpion and triticale seeds of cultivar Raritet with the nitric oxide donor sodium nitroprusside (SNP) on their germination and seedling growth parameters. A three-hour treatment of seeds with SNP (optimum concentration 100 µM) resulted in increased germination energy, germination, and accumulation of shoot and root biomass in both species. The effects of SNP were almost completely eliminated by the action of the NO scavenger methylene blue. Moreover, there was no effect of SNP on seed germination and seedling growth subsequent to its preliminary decomposition under light conditions. This suggests that the physiological activity of SNP may be linked to the release of nitrogen oxide. Treatment of seeds with SNP resulted in a reduction in the generation of superoxide anion radicals and hydrogen peroxide in the shoots of seedlings of both species, as well as a reduction in the content of malonic dialdehyde, a product of lipid peroxidation. Concomitantly, alterations in the activity of pivotal antioxidant enzymes (superoxide dismutase and catalase) were observed in seedlings derived from seeds treated with SNP.

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Furthermore, the application of SNP to seeds resulted in an increase in the total phenolic compound content in wheat seedlings and the amount of anthocyanins in triticale seedlings. It was concluded that there is a correlation between the ability of exogenous NO to mitigate oxidative damage and enhance germination of aged cereal seeds.

Keywords: *Triticum aestivum,* × *Triticosecale*, seed aging, germination, nitric oxide, oxidative stress, antioxidant system

INTRODUCTION

Nitric oxide (NO) is an important gasotransmitter molecule at the centre of the signalling network of plant and animal cells (Sougrakpam *et al*., 2023; Khator *et al*., 2024). Nitric oxide is known to be involved in the regulation of multiple functions of the plant organism: seed germination, flowering, pollen formation, fruit ripening and senescence, as well as legume-rhizobium symbiosis and responses to biotic and abiotic stressors (Turkan, 2017; Venkatesan *et al*., 2020; Singhal *et al*., 2021). The molecular basis for these diverse physiological effects of it is the process of post-translational modification of many proteins, as well as direct and indirect interactions with other key players in the signalling network (primarily reactive oxygen species (ROS) and calcium ions) and many plant hormones (Sami *et al*., 2018; Khan *et al*., 2023; Sougrakpam *et al*., 2023).

One of the important biological effects of nitric oxide, which is not only of fundamental but also of applied importance, is its ability to stimulate the germination of seeds of various plant species. The activation of seed germination of some plant species by inorganic and organic nitrates was discovered at the end of the last century (Grubisic and Konjevic, 1990; Grubisic *et al*., 1992). Later, the effect of NO donors on dormancy interruption and seed germination was studied in detail using the model species *Arabidopsis thaliana* L. (Bethke *et al*., 2004). To date, this phenomenon has been documented in experiments on the effect of nitric oxide donors on seed germination of plants of different taxonomic affiliation, in particular lettuce (*Lactuca sativa* L.), yellow lupin (*Lupinus luteus* L.), tomato (*Solanum lycopersicum* L.), barley (*Hordeum vulgare* L.), millet (*Panicum virgatum* L.) and other cultivated plant species (see reviews: Bethke *et al*., 2007; Krasuska *et al*., 2015). The applied aspect of these studies is mainly related to the search for ways to increase seed germination energy in species whose seeds do not germinate uniformly due to biological peculiarities (Sarath and Mitchell, 2008). The effect of NO donors on seed germination under stress conditions has also been widely studied (Duan *et al*., 2007; Sepehri and Rouhi, 2016). One of the main mechanisms of the beneficial effect of nitric oxide on seed germination is considered to be its modification of the hormonal balance in germinating seeds, associated with the inhibition of abscisic acid synthesis enzymes (the hormone responsible for seed dormancy) under the action of NO and the activation of enzymes providing synthesis of gibberellic acid and ethylene, factors promoting seed germination (Šírová *et al*., 2011; Arc *et al*., 2013; Kolbert *et al*., 2019b, Kolupaev *et al*., 2024b).

To increase the speed and uniformity of seed germination, especially under unfavourable conditions, priming technologies have been used quite actively in the last decade (Janmohammadi, 2012; Paparella *et al.,* 2015; Sako *et al.*, 2020). The term 'seed priming' usually defines a method of controlled wetting and drying of grains to enhance pre-germination metabolic processes in them (Waqas *et al.*, 2019). The simplest priming technique, hydropriming, is more commonly used in combination with seed exposure to solutions of various physiologically active compounds, mainly plant hormones and signalling mediators (Ibrahim, 2019; Sen *et al.,* 2021), including NO (Duan *et al*., 2007; Kaur and Kaur, 2018; Kumar *et al.,* 2021).

Long-term storage is known to reduce seed germination and viability due to ageing. Preservation of seed germination for a certain period depends on both species characteristics and storage conditions. It is known that storing seeds at higher humidity and temperature accelerates ageing, which eventually leads to loss of viability (Probert *et al.*, 2007). One of the causes of accelerated seed ageing under inappropriate storage is the spontaneous formation of ROS in mitochondria and other organelles (Ratajczak *et al.,* 2019) and, as a consequence, lipid peroxidation (LPO), which leads to changes in the composition, structure and function of biomembranes (Kurek *et al*., 2019; Lin *et al.*, 2022). The development of oxidative stress also causes protein carbonylation characteristic of ageing seeds (Rajjou *et al*., 2008) and, in some cases, DNA damage (Kurek *et al.,* 2019; Afzal, 2023).

It has also been shown that the onset of seed germination is often accompanied by an increase in the generation of ROS, which are involved in the generation of redox signals necessary for seedling growth (Kranner *et al*., 2010). However, in ageing seeds there is usually an imbalance between ROS generation and their neutralisation by the antioxidant system (Zhang *et al.,* 2021), which may be responsible for oxidative damage to the structures of the developing seedling. In this regard, it has been suggested that priming aged seeds with antioxidants may improve their germination. In particular, classical antioxidants such as reduced glutathione and ascorbic acid have been shown to improve germination of aged oat seeds (Xia *et al*., 2020). Priming with melatonin, which has direct and indirect antioxidant effects, improved germination of aged rye and triticale seeds (Kolupaev *et al.*, 2024a).

The ability of nitric oxide to alter redox homeostasis is well established (Arnao and Hernández-Ruiz, 2019; Kolbert *et al*., 2019a; Kolupaev *et al.*, 2023a; Dey *et al.,* 2024). Although the mechanisms of such effects are not fully understood, nitric oxide can also have pro-oxidant effects, causing so-called nitrosative stress (Corpas and Barroso, 2013). It is known that, depending on the concentration, nitric oxide can cause various post-translational modifications (nitrosation, tyrosine nitration, metallonitrosylation) in the molecules of antioxidant enzymes, leading to modulation of their activity (Arora *et al*., 2016; Singhal *et al*., 2021; Mukherjee and Corpas, 2023). Nitric oxide also affects the activity of enzymes that regulate the pool of reducing agents (Corpas *et al.*, 2013). Finally, nitric oxide can modulate the activity of the key ROS-generating enzyme, NADPH oxidase (Yun *et al.*, 2011). The direct effect of NO on ROS levels and redox homeostasis is combined with its mediated effect (involving other signalling mediators) on the gene expression of antioxidant enzymes, which usually results in enhanced antioxidant activity of plant cells (Correa-Aragunde *et al.*, 2015; Kolupaev *et al.*, 2023a).

The influence of nitric oxide on seed germination is largely attributed to the modulation of redox homeostasis. Models of an 'oxidative window' and a 'nitrosative door' have been proposed, according to which ROS and reactive nitrogen species (RNS) in a certain concentration range can act as signalling molecules necessary for the induction of metabolic changes responsible for the transition of seeds from dormancy to germination (Krasuska *et al.*, 2015). It is thought that seeds remain dormant at low levels of ROS and RNS in the cells, whereas an increased concentration of ROS and RNS leads to seed senescence (Krasuska *et al.*, 2015).

Nevertheless, there are very few experimental data on the effect of NO donor priming on the germination of aged seeds of cultivated plants and the state of their antioxidant system. In particular, purely phenomenological data have been obtained on the enhancement of germination of millet seeds with reduced germination due to long-term storage by the NO donor sodium nitroprusside (SNP) (Sarath and Mitchell, 2008). It was shown that SNP treatment improved the germination of aged sunflower seeds and reduced the content of the LPO product malonic dialdehyde (MDA), while no significant changes in the activity of antioxidant enzymes were observed (Pereira *et al.*, 2022). It was also shown that treatment of aged oat seeds with SNP increased their germination, which was accompanied by improved functioning of the mitochondrial tricarboxylic acid cycle, increased activity of ascorbate-glutathione cycle enzymes and activation of alternative oxidase (Mao *et al.*, 2018).

However, for seeds of one of the most important crops, bread wheat (*Triticum aestivum* L.), there are only data on SNP-induced enhancement of germination of seeds with normal germination ability against the background of its inhibition by osmotic (Hua *et al.*, 2003), salt (Duan *et al.*, 2007) or cold (Bibi *et al.*, 2020) stress. These effects were accompanied by modulation of antioxidant enzyme activity and a reduction in the manifestation of oxidative stress effects. To date, there is a complete lack of information on the effect of NO donors on the germination of triticale seeds, a hybrid species obtained by crossing wheat and rye and characterised by a rather rapid decrease in seed germination when stored under suboptimal conditions (Kolupaev *et al.*, 2024b).

In this context, the aim of the work was to study the effect of the nitric oxide donor SNP on the germination of wheat and triticale seeds subjected to natural ageing during storage under uncontrolled conditions, and the relationship between the physiological effects of exogenous NO and changes in the state of the antioxidant system of germinating seeds.

MATERIAL AND METHODS

Plant materials and treatments

The experiments were carried out in the Laboratory of Plant Physiology and Biochemistry of the Yuriev Plant Production Institute of NAAS of Ukraine. Seed samples were provided by the National Center for Genetic Resources of Plants of Ukraine. Seeds of winter bread wheat (*Triticum aestivum* L.) of the Scorpion cultivar (Czech Republic, Austria) and winter triticale (× *Triticosecale* Wittmack) of the Raritet cultivar (Ukraine) of the 2020 generation were used for the experiments. The wheat cultivar Scorpion has blue grains with increased content of polyphenolic compounds (Martinek *et al*., 2013). However, it is reported that the seeds of this cultivar, even when properly stored, shrink and shrivel easily, resulting in reduced germination (Martinek *et al*., 2013). Triticale seeds of the Raritet cultivar also lose germination significantly after several years of storage, as shown by previously obtained data (Kolupaev *et al.*, 2024a). Prior to the experiments, wheat and triticale seeds were stored indoors for three years under uncontrolled conditions (in summer the temperature periodically reached 30–32°C, in winter it dropped to -6...-8°C; relative humidity during storage repeatedly changed from 25–30 to 80–85%). As a result, seed germination during 3 years of storage decreased to about 50% for wheat and 40% for triticale.

Seeds from all experimental variants were disinfected with 5% sodium hypochlorite solution for 15 min and washed repeatedly with sterile distilled water. Some seeds were then kept in glasses with distilled water for 3 hours (hydropriming). Hydropriming has previously been shown to increase seed germination slightly (approximately within 10%) (Kolupaev *et al.,* 2024a). Therefore, the hydropriming variant was considered as the control variant.

In the SNP treatment variants, seeds were incubated for 3 h in solutions at concentrations of 20, 100, and 500 μM in diffused light at 24°C. In a separate series of experiments to demonstrate the specific effect of SNP as a nitric oxide donor on seed germination, a so-called "exhausted" SNP solution (concentration 100 μM), containing the degradation products of this compound but having lost NO, was used for comparison. Such an "exhausted" SNP solution was obtained by incubating it in an open dish in the light for 2 days (Kolupaev *et al.,* 2020a). In addition, variants with seed treatment with the NO scavenger methylene blue (Zhang *et al.,* 2006) and a mixture of SNP with methylene blue were included in the experiment. In these experimental variants, the seed priming time was also 3h.

Seeds hydroprimed or treated with SNP or other compounds mentioned above were dried in a dark thermostat at 24°C and 40% humidity for one day. The seeds were then placed in Petri dishes with two layers of sterile filter paper moistened with distilled water and germinated in a dark thermostat at 24°C for 3 days. After 2 days of germination, the relative number of germinated seeds (germination energy index) and the mass of shoots and roots of seeds with normal germination were evaluated. On the 3rd day of germination, the germination index (relative number of germinated seeds) was evaluated. The shoots from normally germinated seeds were used for biochemical analyses.

Superoxide anion radical generation

The generation of superoxide anion radicals (SAR) by the shoots was estimated by nitroblue tetrazolium (NBT) reduction. Ten identical shoots were placed for 1 h in a tube containing 5 ml of 0.1 M K, Na-phosphate buffer (pH 7.6) with 0.05% NBT, 10 μM EDTA, and 0.1% Triton X-100 (Kolupaev *et al.*, 2023b). At the end of the exposure, the shoots were gently removed from the incubation solution. The optical density of the incubation solution was also measured at 530 nm using a UV-1280 spectrophotometer (Shimadzu, Japan). The rate of $O_2^{\bullet-}$ generation was calculated in standard units (A_{530} • 1000/weight of shoot raw material).

Evaluation of hydrogen peroxide content

For the determination of H_2O_2 content, seedling shoots were homogenised in cold with 5% trichloroacetic acid (TCA). Samples were centrifuged at 8000×g for 10 min at 2-4°C in an MPW 350R centrifuge (MPW MedInstruments, Poland). The concentration of H_2O_2 in the supernatant was measured by the ferrothiocyanate method (Sagisaka, 1976) with slight modifications. For this purpose, 0.5 ml of 2.5 M ammonium thiocyanate, 0.5 ml of 50% TCA, 1.5 ml of supernatant and 0.5 ml of 10 mM ferrous ammonium sulphate were added to tubes. After mixing, the samples were transferred to cuvettes and the absorbance at 480 nm was determined.

Measurement of LPO products content

For the analysis of the amount of LPO products reacting with 2-thiobarbituric acid (mainly malonic dialdehyde, MDA), shoots were homogenised in a reaction medium containing 0.25% 2-thiobarbituric acid in 10% TCA and the homogenate was placed in tubes with foil lids in a boiling bath for 30 min. The samples were then cooled and centrifuged at 10000×g for 15 min. The absorbance of the supernatant was determined at 532 nm (maximum light absorption of MDA) and 600 nm (to correct for non-specific light absorption) (Kolupaev *et al.*, 2020b).

Antioxidant enzyme activity assessment

Seedling samples were homogenised in cold 0.15 M K, Na-phosphate buffer (pH 7.6) with the addition of EDTA (0.1 mM) and dithiothreitol (1 mM). The homogenate was centrifuged at 8000×g for 15 min at a temperature of not more than 4°C to prepare the supernatant, which was then assayed (Kolupaev *et al.*, 2022a). Superoxide dismutase (SOD) (EC 1.15.1.1) activity was determined at pH 7.6 by a method based on the ability of the enzyme to compete with nitroblue tetrazolium for superoxide anions generated by the aerobic interaction between NADH and phenazine methosulphate. Catalase (EC 1.11.1.6) was evaluated by the amount of H_2O_2 decomposed per unit time. Guaiacol peroxidase (EC 1.11.1.7) activity was estimated at pH 6.2 using guaiacol as hydrogen donor and H_2O_2 as substrate.

Determination of phenolic and anthocyanin content

For the determination of total phenolic compounds and anthocyanins, seedlings (300 mg) were homogenised in 6 ml of 80% ethanol. The extraction was carried out at room temperature for 20 min. The samples were centrifuged at

8,000 × g for 15 min. To evaluate the content of phenolic compounds, 0.5 ml of the supernatant, 8 ml of distilled water and 0.5 ml of Folin's reagent were added to the reaction tubes and stirred; 3 min later, 1 ml of 10% sodium carbonate was added. After 1 hour, the absorbance of the reaction mixture was measured at 725 nm (Bobo-García *et al.,* 2015). The content of phenolic compounds was expressed as μmol gallic acid/g dry weight.

Before the determination of anthocyanin content, the supernatants were acidified with HCl to a final concentration of 1%. Absorbance was measured at 530 nm (Nogués and Baker, 2000). The results were expressed as A_{530}/g dry weight.

Replication of experiments and statistical processing of data

To determine the impact of seed treatment with SNP and other compounds on seed germination and seedling biomass, each experimental variant was replicated at least three times, with each replicate consisting of 60 seeds. For biochemical analyses, each sample consisted of at least 12 seedlings, with analyses performed in three or more replicates.

The statistical analysis of the results was conducted using the analysis of variance (ANOVA) and Fisher’s least significant difference (LSD) test. The figures and table present the mean values from three biological replicates, along with their standard errors. Different letters indicate values that are significantly different at the $P \leq 0.05$ level.

RESULTS AND DISCUSSION

Effect of SNP on seed germination and seedling growth parameters

The germination rates of wheat and triticale seeds exposed to temperature and humidity variations during 3 years of storage were 51.4 and 44.3%, respectively, in the control (Figure 1A). SNP priming in the concentration range of 20–500 μM caused an increase in germination energy of seeds of both species. The maximum effect was observed when seeds were primed with 100 μM SNP. A significant increase in seed germination index after SNP treatment was also observed across the range of concentrations used, with a maximum at 100 μM (Figure 1A).

Seed treatment with SNP also resulted in an increase in biomass accumulation of shoots and especially roots of seedlings (Figure 1B). Thus, in wheat, an increase in root mass of 50–90% relative to the control was observed with SNP treatment at different concentrations. A slightly smaller but quite significant increase in root weight was observed in triticale when seeds were treated with SNP, with an increase of 15-45% at different concentrations. Shoot weight in wheat seedlings was significantly increased by SNP concentrations of 100 and 500 μM (Figure 1B).

In triticale, a significant effect on shoot weight increase was only observed for the 100 μM concentration. In general, seedling weight in wheat increased as a result of SNP priming at all concentrations used, while in triticale this effect was significant for SNP concentrations of 20 and 100 μM (Figure 1B).

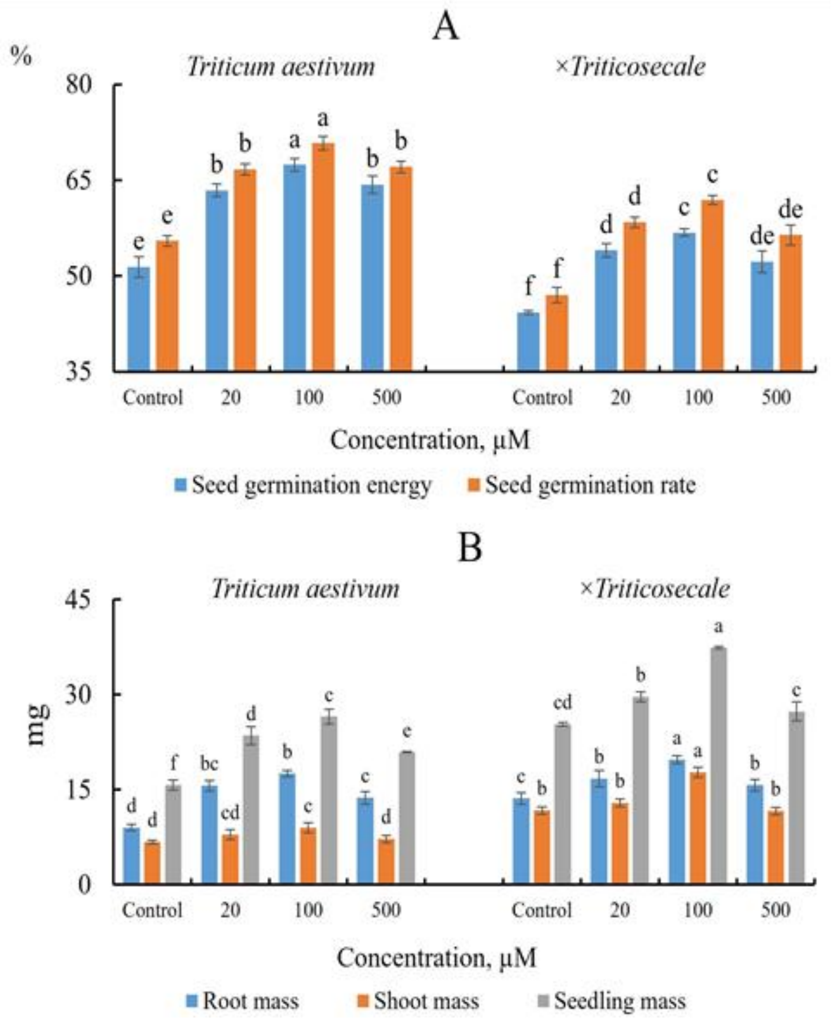
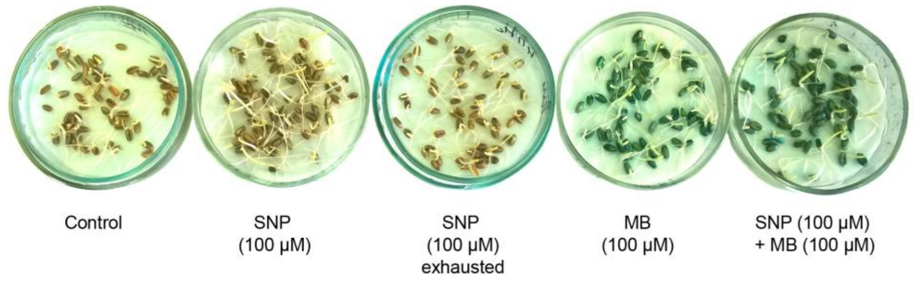


Figure 1. Concentration dependence of the effect of SNP priming on germination energy, seed germination rates (A) and organ biomass (B) of wheat and triticale seedlings

The specificity of the action of SNP as a nitric oxide donor rather than a complex compound is usually confirmed by the use of the so-called 'exhausted' SNP solution, which, as mentioned in the Materials and Methods section, is obtained by decomposition of this salt in the light (Mur *et al.*, 2013). As our results showed, the 'exhausted' SNP at a concentration corresponding to the most effective concentration of freshly prepared SNP (100 μM) had no significant effect on any of the parameters characterising seed germination and seedling growth in wheat (Figure 2, Table 1). In triticale, no significant effect of the 'exhausted' SNP on germination energy and germination indices was found. At the same time, when seeds were treated with the 'exhausted' SNP, a slight increase in root weight and, as a consequence, in total seedling weight was observed (Table 1). Thus, we can speak of a small non-specific growth stimulating effect of the 'exhausted' SNP when triticale seeds were treated with it. However, these effects were much smaller compared to the effects of freshly prepared SNP.

Triticum aestivum



×*Triticosecale*

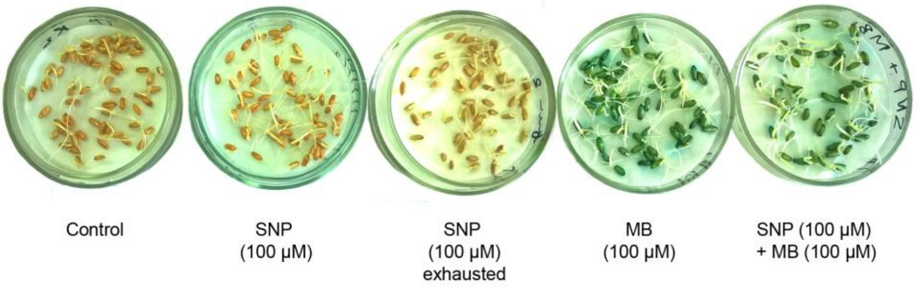


Figure 2. Condition of wheat and triticale seedlings when seeds were treated with SNP, 'exhausted' SNP and the NO scavenger methylene blue (MB)

Another way of demonstrating the specificity of the effect of SNP as an NO donor is to use it in combination with nitric oxide scavengers. In our experiments, treatment of wheat seeds with SNP in combination with methylene blue completely abolished the effect of the nitric oxide donor on all the indicators of seed germination and seedling growth studied (Table 1).

The combined effect of SNP and methylene blue on triticale seeds did not show the effect of NO donor on germination energy, although the germination index in the variant with the combination of SNP and methylene blue was slightly higher than in the control, but significantly lower than in the variant with priming seeds with SNP alone (Table 1). Seed treatment with methylene blue also completely eliminated the effect of SNP on seedling growth parameters.

It should be noted that in the variant with priming of wheat seeds with methylene blue only, a significant decrease in germination energy and germination was observed, although the growth of seedling organs in this variant did not differ from the control. In triticale under the influence of seed priming with methylene blue, a significant decrease in seed germination and root biomass was recorded (Table 1). In general, treatment of wheat and triticale seeds with methylene blue slightly worsened their germination, indicating the role of endogenous nitric oxide in the germination of seeds of these cereal species.

Table 1. Modification of the SNP effect on seed germination and morphometric indices of seedlings with its pre-light treatment and combination with methylene blue (MB)

Variant	Germination energy, %	Seed germination rates, %	Root weight, mg	Shoot weight, mg	Seedling weight, mg
Triticum aestivum L.					
Control	49.7±1.0 cd	53.6±1.2 c	10.0±0.6 f	6.7±0.3 d	16.7±0.8 de
SNP 100 µM	64.5±1.1 a	69.8±1.2 a	16.4±0.9 b	9.3±0.4 c	25.7±0.8 bc
SNP 100 µM 'exhausted'	52.2±0.4 c	55.5±1.4 c	10.2±0.9 f	7.0±0.7 d	17.2±0.3 de
SNP (100 µM) + MB (100 µM)	44.7±1.5 de	46.7±1.1 de	10.0±0.3 f	7.7±0.7 d	17.7±0.4 d
MB (100 µM)	43.2±1.5 e	46.3±0.9 de	8.6±0.4 f	7.1±0.3 d	15.7±0.1 e
×*Triticosecale*					
Control	45.7±1.7 de	48.4±0.7 d	12.5±0.5 d	12.0±0.2 b	24.5±0.4 c
SNP 100 µM	57.7±0.7 b	61.2±0.8 b	18.7±0.7 a	16.7±1.0 a	35.4±1.7 a
SNP 100 µM 'exhausted'	44.3± 0.9 e	47.7±1.5 d	14.7±0.6 c	12.7±0.8 b	27.4±0.2 b
SNP (100 µM) + MB (100 µM)	47.3±1.0 d	53.1±1.6 c	11.8±0.6 de	12.8±0.9 b	24.7±0.3 c
MB (100 µM)	42.2±1.1 e	44.6±1.6 e	13.1±0.3 d	10.7±0.7 c	23.8±0.4 c

Amooaghaie and Nikzad (2013) showed that methylene blue eliminated the enhancement of tomato seed germination induced by SNP treatment under low temperature conditions. Another NO scavenger, cPTIO (2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide), inhibited seed germination of the woody plant Paulownia elongata, which is characterised by physiological dormancy (Liu *et al.*, 2019). Thus, our data on the retardation of germination of aged wheat and triticale seeds in the presence of methylene blue and its abolition of the positive effect of SNP on seed germination are consistent with data on the role of endogenous and exogenous NO in the interruption of seed dormancy obtained for other plant species.

Effect of SNP on ROS generation and MDA levels in wheat and triticale seedlings

When studying the effect of nitric oxide donor on indicators characterising the level of oxidative stress during seed germination, it was used at the concentration that had the most significant effect on seed germination and seedling growth, 100 μM. Under the influence of SNP, a significant decrease in the generation of superoxide anion radicals was observed in wheat seedlings and even more significantly in triticale (Figure 3A). Hydrogen peroxide content was also reduced under the influence of SNP seed priming in shoots of seedlings of both species, but this effect was more pronounced in triticale seedlings (Figure 3B). The amount of the LPO product MDA in SNP-primed variants decreased in both cereal species, but more significantly in wheat (Figure 3C).

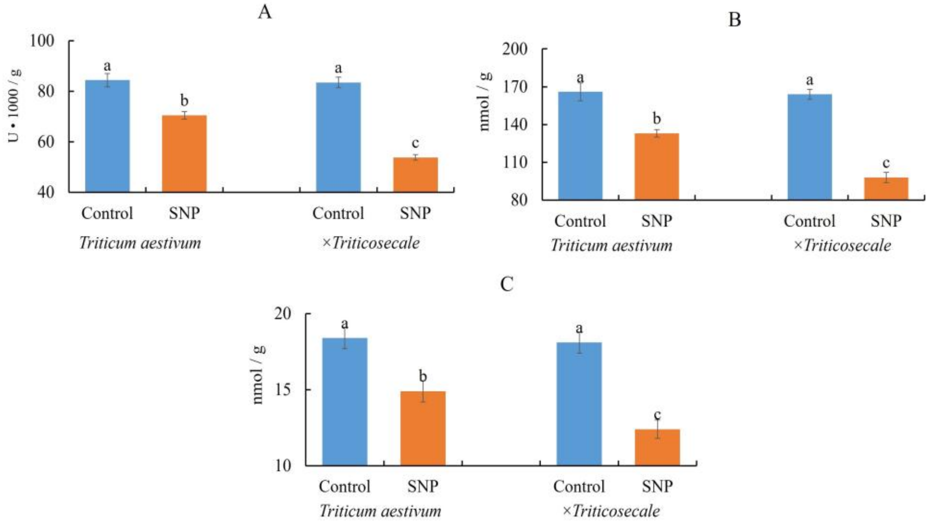


Figure 3. Effect of seed SNP priming on superoxide anion radical generation (A), hydrogen peroxide (B) and MDA (C) content in wheat and triticale seedlings

In general, the treatment of wheat and triticale seeds resulted in a reduction in all the studied parameters that characterise the level of oxidative stress. In this context, the influence of SNP on the functioning of the antioxidant system was studied in subsequent experiments.

Effect of SNP seed priming on antioxidant enzyme activity in wheat and triticale seedlings

SOD activity was higher in wheat seedlings (Figure 4A). At the same time, SNP treatment caused its decrease. In triticale seedlings, this index also decreased under the influence of SNP. At the same time, there is evidence in the literature about the positive modulation of SOD activity by nitric oxide donors due to the corresponding post-translational modification (Sougrakpam *et al*., 2018; Pereira *et al.,* 2022). It should be noted that in our experiments, the decrease in SOD activity in the nitric oxide donor seed treatment variant in seedlings of both cereal species was accompanied by a concomitant decrease in superoxide anion radical generation. A possible explanation for this apparently paradoxical effect could be suppression of NADPH oxidase activity by nitric oxide due to post-translational modification of the enzyme (Yun *et al*., 2011). However, further studies are needed to clarify the interpretation of these results.

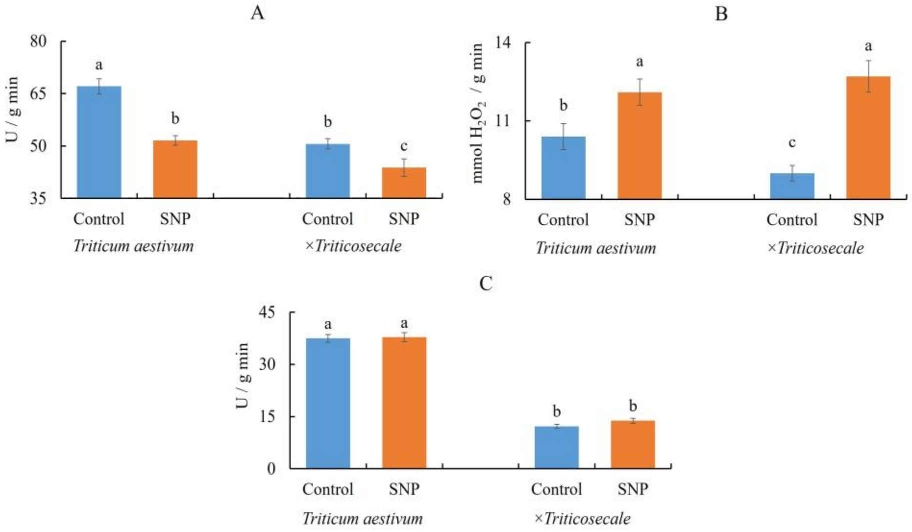


Figure 4. Effect of seed SNP priming on SOD (A), catalase (B) and guaiacol peroxidase (C) activity in wheat and triticale seedlings

SNP priming of seeds of both cereal species resulted in an increase in catalase activity (Figure 4B). This effect is consistent with data obtained when SNP priming of aged sunflower seeds was studied (Pereira *et al.*, 2022). SNP priming of peanut seeds also promoted catalase activity during germination under osmotic stress conditions (Sepehri and Rouhi, 2016). The effects of increased catalase activity under the influence of NO donor have been attributed to S-nitrosylation of specific cysteine residues (Sougrakpam *et al.*, 2018). However, specific studies are also needed to assess the contribution of this mechanism to the regulation of catalase activity under specific experimental conditions.

The activity of guaiacol peroxidase in the control variant was much lower in triticale seedlings than in wheat seedlings (Figure 4C). As shown by studies with other varieties of these cereals, low guaiacol peroxidase activity is a species characteristic of triticale (Kolupaev *et al.*, 2022b). However, under the conditions of our experiments, SNP had no effect on enzyme activity in seedlings of either cereal species. It is known that non-specific peroxidase (guaiacol peroxidase) can not only neutralise ROS, but also participate in ROS generation, i.e. exhibit pro-oxidant effects (Kolupaev and Karpets, 2014). Therefore, an increase in peroxidase activity is not always a sign of activation of antioxidant processes. At the same time, catalase, whose activity was increased by SNP seed treatment, effectively fulfils the functions of neutralising high concentrations of H_2O_2 (Das and Roychoudhury, 2014). In this context, the increase in its activity in the absence of changes in the activity of guaiacol peroxidase in seedlings from SNP-primed seeds can be considered as the reason for the marked decrease in their hydrogen peroxide content.

Effect of SNP seed treatment on the content of secondary metabolites in seedlings

It is established that secondary metabolites, namely phenolic and polyphenolic (flavonoid) compounds, play a pivotal role in the functioning of the antioxidant system of cereals (Kolupaev *et al.*, 2023c). In this context, we investigated the effect of SNP on the content of phenolics and anthocyanins in shoots of wheat and triticale seedlings.

SNP priming of wheat seeds caused an increase in the total phenolic content (Figure 5A), while the anthocyanin content did not change (Figure 5B). A different pattern was observed in triticale seedlings derived from SNP-primed seeds. They showed a significant increase in the content of anthocyanins with no significant changes in the total content of phenolic compounds (Figure 5). It should be noted that the high basal content of anthocyanins and its increase in response to adverse factors is a species characteristic of triticale and distinguishes it from most wheat varieties (Kolupaev *et al*., 2020b; 2022b). At the same time, as mentioned above, the blue-grained wheat variety Scorpion used in our work is characterised by a high content of phenolic compounds in general (Martinek *et al.*, 2013). Thus, their amount in etiolated seedlings was the same as in triticale seedlings (Figure 5). It can be assumed that the increase in phenolic compounds in wheat and flavonoids (anthocyanins) in triticale during seed priming with a nitric oxide donor contributed to the reduction of superoxide radical generation (Figure 3A) and possibly other radical ROS during seed germination. Secondary metabolites, and anthocyanins in particular, are known to be effective in neutralising ROS, primarily radical forms, including the superoxide anion radical (Neill and Gould, 2003).

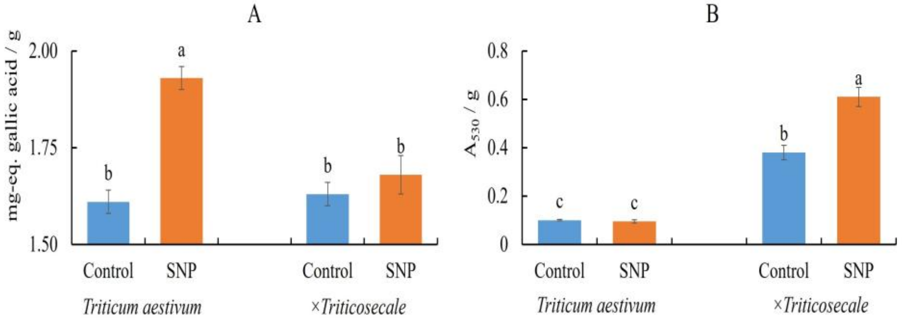


Figure 5. Effect of seed SNP priming on the content of phenolic compounds (A) and anthocyanins (B) in shoots of wheat and triticale seedlings

Possible relationship between the effect of nitric oxide donor on redox homeostasis and seed germination

In general, the priming of old wheat and triticale seeds, which promoted their germination and seedling growth, significantly modified the studied indicators of the pro/antioxidant balance (Figure 6). The levels of all markers of oxidative stress decreased in the seedlings of both cereal species: superoxide anion radical generation, hydrogen peroxide and MDA content. In the context of reduced O_2•– formation under the action of NO donor, SOD activity decreased in both species. At the same time, the activity of the key hydrogen peroxide decomposing enzyme, catalase, increased in both species when seeds were treated with SNP. In the absence of changes in guaiacol peroxidase activity, it can be assumed that catalase was the major contributor to the regulation of hydrogen peroxide levels in seedlings. Secondary metabolites, such as various phenolic compounds in wheat and anthocyanins in triticale, also appeared to contribute to the regulation of the levels of different ROS during SNP treatment (Figure 6).

Undoubtedly, the above explanation of the effect of nitric oxide donors on ROS generation and the state of the antioxidant system in seedlings of the studied cereals is very schematic and simplified. According to modern models, nitric oxide can exert a complex influence on the pro/antioxidant balance in plant cells. It involves post-translational modification of proteins (including directly antioxidant enzyme molecules), which can lead to both increased and decreased activity, depending on the type of modification (S-nitrosation or tyrosine nitration) (Aroca *et al*., 2015; Correa-Aragunde *et al*., 2015; Corpas *et al.*, 2019). Nitric oxide can also modulate the activity of pro-oxidant enzymes such as NADPH oxidase (Yun *et al.*, 2011). In addition, NO affects enzymes responsible for maintaining the pool of reducing agents (Aroca *et al.*, 2015; Corpas *et al.*, 2021), which may also alter the pro/antioxidant balance in cells. Finally, through its involvement in the regulatory network, nitric oxide, together with ROS, can influence gene expression of antioxidant enzymes (Kolupaev *et al*., 2023a).

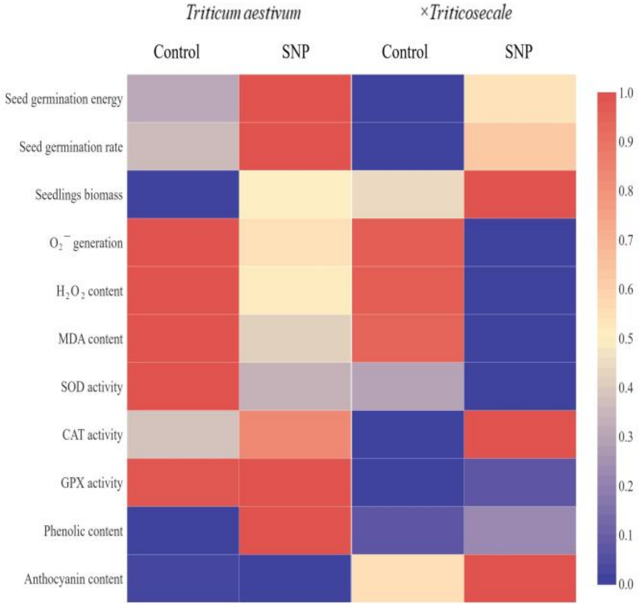


Figure 6. Heat map of changes in growth parameters and antioxidant system of wheat and triticale seedlings under the influence of 100 μM SNP. All values are normalised from 0 to 1

However, it is difficult to interpret the mechanisms of nitric oxide influence on the antioxidant system of seedlings after seed priming because it is not clear how long the effect of SNP lasts after its treatment. At the same time, the lack of effect of SNP on seed germination and seedling growth after light treatment or when used in combination with the nitric oxide scavenger methylene blue indicates the key role of NO in the physiological effects of SNP (Table 1).

CONCLUSIONS

The results obtained indicate that priming aged wheat and triticale seeds with the nitric oxide donor SNP can be an effective tool for increasing their germination and improving seedling growth. At the same time, an important component of the SNP effect is the alleviation of oxidative stress effects during germination of aged cereal seeds (reduced generation of superoxide anion radical and hydrogen peroxide, decreased malondialdehyde content). Seed treatment with SNP modulated the activity of antioxidant enzymes SOD and catalase in cereal seedlings, and also enhanced the accumulation of secondary metabolites with antioxidant properties.

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REFERENCES

Afzal, I. (2023). Seed priming: what's next? Seed Science and Technology, 51(3): 379-405.

Amooaghaie, R, & Nikzad, K. (2013). The role of nitric oxide in priming-induced low-temperature tolerance in two genotypes of tomato. Seed Science Research, 23(2):123-131.

Arc, E., Sechet, J., Corbineau, F., Rajjou, L., & Marion-Poll, A. (2013). ABA crosstalk with ethylene and nitric oxide in seed dormancy and germination. Frontiers in Plant Science, 4:63. https://doi.org/10.3389/fpls.2013.00063

Arnao, M. B., & Hernández-Ruiz, J. (2019). Melatonin and reactive oxygen and nitrogen species: a model for the plant redox network. Melatonin Research, 2:152-168.

Aroca, A., Serna, A., Gotor, C., and Romero, L. C. (2015). S-sulfhydration: A cysteine posttranslational modification in plant systems. Plant Physiology, 168:334-342.

Arora, D., Jain P., Singh N., Kaur H. & Bhatla S. C. (2016). Mechanisms of nitric oxide crosstalk with reactive oxygen species scavenging enzymes during abiotic stress tolerance in plants. Free Radical Research, 50:291-303.

Bethke, P. C., Badger, M. R., & Jones, R. L. (2004). Apoplastic synthesis of nitric oxide by plant tissues. The Plant Cell, 16(2):332-341.

Bethke, P. C., Libourel, I. G. L., & Jones, R. L. (2007). Nitric oxide in seed dormancy and germination. Annual Review of Plant Biology, 27:153-175.

Bibi, A., Majid, S.A., Azhar, N., Amjad, M.S., Ashraf, S., Ahmad, I., Mumtaz, S., & Ijaz, S. (2020). Differential changes in growth and enzyme activities of chilling induced wheat seedlings by nitric oxide priming. International Journal of Agriculture and Biology, 23:919-926.

Bobo-García, G., Davidov-Pardo, G., Arroqui, C., Vírseda, P., Marín-Arroyo, M. R., & Navarro, M. (2015). Intra-laboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts, and comparison with conventional spectrophotometric methods. Journal of the Science of Food and Agriculture, 95(1):204-209.

Corpas, F. J., & Barroso, J. B. (2013). Nitro-oxidative stress vs oxidative or nitrosative stress in higher plants. New Phytologist, 199:633-635.

Corpas, F. J., González-Gordo, S., & Palma, J. M. (2021). Nitric oxide and hydrogen sulfide modulate the NADPH-generating enzymatic system in higher plants. Journal of Experimental Botany, 72 (3):830-847.

Corpas, F. J., González-Gordo, S., Canas, A., & Palma, J. M. (2019). Nitric oxide (NO) and hydrogen sulfide (H2S) in plants: Which is first? Journal of Experimental Botany, 70 (17):4391-4404.

Correa-Aragunde, N., Foresi, N., & Lamattina, L. (2015). Nitric oxide is a ubiquitous signal for maintaining redox balance in plant cells: Regulation of ascorbate peroxidase as a case study. Journal of Experimental Botany, 66(10):2913-2921.

Das, K. & Roychoudhury, A. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in Environmental Science, 2:53. https://doi.org/10.3389/fenvs.2014.00053

Dey, P., Pattanaik, D., Mohapatra, D., Saha, D., Dash, D., Mishra, A., Priyadarshinee, L., Singh, A., Swain, P., Baig, M.J., Kherawat, B.S., Chung, S.-M., Kumar, M., Badu, M., Singhal, R. K., Gaikwad, D., Khan, M. N., Manohar, S. & Kesawat, M. S. (2024). Gasotransmitters signaling and their crosstalk with other signaling molecules under diverse stress conditions in plants. South African Journal of Botany, 169:119-133.

Duan, P., Ding, F., Wang, F., & Wang, B.S. (2007). Priming of seeds with nitric oxide donor sodium nitroprusside (SNP) alleviates the inhibition on wheat seed germination by salt stress. Journal of Plant Physiology and Molecular Biology, 33:244-250.

Grubisic, D., & Konjevic, R. (1990). Light and nitrate interaction in phytochrome-controlled germination of Paulownia tomentosa seeds. Planta, 181:239-243.

Grubisic, D., Giba, Z., & Konjevic, R. (1992). The effect of organic nitrates in phytochrome-controlled germination of Paulownia tomentosa seeds. Photochemistry and Photobiology, 56:629-632.

Hua Z., Shen W.-B. & Xu L.-L. (2003). Effects of nitric oxide on the germination of wheat seeds and its reactive oxygen species metabolisms under osmotic stress. Acta Botanica Sinica, 45:901-905.

Ibrahim, E.A.-A. (2019). Fundamental processes involved in seed priming. In: Hasanuzzaman M., Fotopoulos V. (eds.), Priming and Pretreatment of Seeds and Seedlings (Springer Nature Singapore Pte Ltd.) 63-115.

Janmohammadi M. (2012): Alleviation the adverse effect of cadmium on seedling growth of greater burdock (Aractium lappal L.) through pre-sowing treatments. Agriculture and Forestry, 56 (1-4): 55-70.

Kaur, K., & Kaur, K. (2018). Nitric oxide improves thermotolerance in spring maize by inducing varied genotypic defense mechanisms. Acta Physiologiae Plantarum, 40:55. https://doi.org/10.1007/s11738-018-2632-9

Khan, M., Ali, S., Al Azzawi, T. N. I., & Yun, B.-W. (2023). Nitric oxide acts as a key signaling molecule in plant development under stressful conditions. International Journal of Molecular Sciences, 24:4782. https://doi.org/10.3390/ijms24054782

Khator, K., Parihar, S., Jasik, J., & Shekhawat, G. S. (2024). Nitric oxide in plants: an insight on redox activity and responses toward abiotic stress signaling. Plant Signaling & Behavior, 19(1). https://doi.org/10.1080/15592324.2023.2298053

Kolbert, Z. S., Barroso, J. B., Brouquisse, R., Corpas, F. J., Gupta, K. J., Lindermayr, C., Loake, G. J., Palma, J. M., Petrivalský, M., & Wendehenne, D. (2019a). A forty year journey: The generation and roles of NO in plants. Nitric Oxide, 93:53-70.

Kolbert, Z., Feigl, G., Freschi, L., Poór, P. (2019b). Gasotransmitters in action: Nitric Oxide-ethylene crosstalk during plant growth and abiotic stress responses. Antioxidants, 8:167. https://doi.org/10.3390/antiox8060167

Kolupaev Y. E., & Karpets, Y. V. (2014). Reactive oxygen species and stress signaling in plants. Ukrainian Biochemical Journal, 86(4):18-35. https://doi.org/10.15407/ubj86.04.018

Kolupaev, Y. E., Horielova, E. I., Yastreb, T. O. Ryabchun, N. I. & Reznik A. M. (2020a). Nitrogen oxide donor enhances cold-induced changes in antioxidant and osmoprotective systems of cereals. Applied Biochemistry and Microbiology, 56(2):219-225.

Kolupaev, Y. E., Horielova, E. I., Yastreb, T. O., Ryabchun, N. I. (2020b). State of antioxidant system in triticale seedlings at cold hardening of varieties of different frost resistance. Cereal Research Communications, 48(2):165-171.

Kolupaev Yu. E., Yastreb T. O., Salii A. M., Kokorev A. I., Ryabchun N. I., Zmiievska O. A., & Shkliarevskyi M. A. (2022a). State of antioxidant and osmoprotective systems in etiolated winter wheat seedlings of different cultivars due to their drought tolerance. Žemdirbystė - Agriculture, 109(4):313-322.

Kolupaev, Y. E., Shkliarevskyi, M. A., Pyshchalenko, M. A., Dmitriev, A. P. (2024b). Nitric oxide: functional interaction with phytohormones and applications in crop production. Agriculture and Forestry, 70(1):379-411. https://doi.org/10.17707/AgricultForest.70.1.24

Kolupaev, Y. E., Taraban, D. A., Kokorev, A. I., Yastreb, T. O., Pysarenko, V. M., Sherstiuk E., & Karpets Y. V. (2024a). Effect of melatonin and hydropriming on germination of aged triticale and rye seeds. Botanica, 30(1):1-13. https://doi.org/10.35513/Botlit.2024.1.1

Kolupaev, Y. E., Yemets, A. I., Yastreb, T. O. & Blume, Y. B. (2023a). The role of nitric oxide and hydrogen sulfide in regulation of redox homeostasis at extreme temperatures in plants. Frontiers in Plant Science, 14:1128439. https://doi.org/10.3389/fpls.2023.1128439

Kolupaev, Y. E., Makaova B. E., Yastreb T. O., Ryabchun N. I., Tyshchenko V. M., Barabolia O. V., & Shkliarevskyi M. A. (2023b). Growth responses of wheat seedlings of different varieties to heat-stress and their relation to the antioxidant system state and osmolytes accumulation. Biologichni Studii - Studia Biologica. 17(1):81-97.

Kolupaev, Y. E., Makaova, B. E., Ryabchun, N. I., Kokorev, A. I., Sakhno, T. V., Sakhno, Y., Yastreb, T. O., & Marenych, M. M. (2022b). Adaptation of cereal seedlings to oxidative stress induced by hyperthermia. Agriculture and Forestry, 68 (4):7-18.

Kolupaev, Y. E., Yastreb, T. O., Ryabchun, N. I., Kokorev A. I., Kolomatska V. P. & Dmitriev A. P. (2023c). Redox homeostasis of cereals during acclimation to drought. Theoretical and Experimental Plant Physiology, 35(2):133-168.

Kranner, I., Minibayeva, F. V., Beckett, R. P., Seal, C. E. (2010). What is stress? Concepts, definitions and applications in seed science. New Phytologist, 188(3):655-673.

Krasuska, U., Ciacka, K., Andryka, P., Bogatek, R., & Gniazdowska, A. (2015). “Nitrosative door” in seed dormancy alleviation and germination. In: Gupta K.J., Igamberdiev A.U. (eds.) Reactive Oxygen and Nitrogen Species Signaling and Communication in Plants (Springer International Publishing: Cham, Switzerland) 215-237.

Kumar, V., Dwivedi, P., Kumar, P., Singh, B. N., Pandey, D. K., Kumar, V., & Bose, B. (2021). Mitigation of heat stress responses in crops using nitrate primed seeds. South African Journal of Botany, 140:25-36.

Kurek, K., Plitta-Michalak, B., & Ratajczak, E. (2019). Reactive oxygen species as potential drivers of the seed aging process. Plants, 8(6):174. https://doi.org/10.3390/plants8060174

Lin, Y. X., Xu, H. J., Yin, G. K., Zhou, Y. C., Lu, X. X., & Xin, X. (2022). Dynamic changes in membrane lipid metabolism and antioxidant defense during soybean (Glycine max L. Merr.) seed aging. Frontiers in Plant Science, 13:908949. https://doi.org/10.3389/fpls.2022.908949

Liu, J., Xue, T., & Shen, Y. (2019). Effect of nitric oxide on seed germination and dormancy in empress trees. HortTechnology, 29(3):271-275.

Mao, C., Zhu, Y., Cheng, H., Yan, H., Zhao, L., Tang, J., Ma, X., & Mao, P. (2018). Nitric Oxide regulates seedling growth and mitochondrial responses in aged oat seeds. International Journal of Molecular Sciences, 19(4):1052. https://doi.org/10.3390/ijms19041052

Martinek, P., Skorpik, M., Chrpova, J., & Schweiger, J. (2013). Development of the new winter wheat variety Skorpion with blue grain. Czech Journal of Genetics and Plant Breeding, 49:90-94.

Mukherjee, S., & Corpas, F. J. (2023). H2O2, NO, and H2S networks during root development and signalling under physiological and challenging environments: Beneficial or toxic? Plant, Cell & Environment, 46(3):688-717.

Mur, L. A. J., Mandon, J., Persijn, S., Cristescu, S. M., Moshkov, I. E., Novikova, G. V., Hall, M. A., Harren, F. J. M., Hebelstrup, K. H., & Gupta K. J. (2013). Nitric oxide in plants: an assessment of the current state of knowledge. AoB Plants, 5:Pls052. https://doi.org/10.1093/aobpla/pls052

Neill S. O., & Gould K. S. (2003). Anthocyanins in leaves: light attenuators or antioxidants? Functional Plant Biology, 30(8): 865. https://doi.org/10.1071/fp03118

Nogués S., & Baker N. R. (2000). Effects of drought on photosynthesis in Mediterranean plants grown under enhanced UV-B radiation. Journal of Experimental Botany, 51(348):1309-1317. https://doi.org/10.1093/jxb/51.348.1309

Paparella, S., Araújo, S. S., Rossi, G., Wijayasinghe, M., Carbonera, D., & Balestrazzi, A. (2015). Seed priming: state of the art and new perspectives. Plant Cell Reports, 34(8):1281-1293.

Pereira, A. A. S., Nery, F. C., Ferreira, R. A., Silva, V. N. D., Bernardes, M. M., Santos, H. O. D., & Bicalho, E. M. (2022). Can priming with ascorbic acid or nitric oxide improve the germinability of stored sunflower seeds?. Journal of Seed Science, 44:e202244012. https://doi.org/10.1590/2317-1545v44256600

Probert, R., Adams, J., Coneybeer, J., Crawford, A., & Hay, F. 2007. Seed quality for conservation is critically affected by pre-storage factors. Australian Journal of Botany, 55:326–335.

Rajjou, L., Lovigny, Y., Groot, S.P.C., Belghaz, M., Job, C., & Job. D. (2008). Proteome-wide characterization of seed aging in Arabidopsis: a comparison between artificial and natural aging protocols. Plant Physiology, 148:620-641.

Ratajczak, E., Małecka, A., Ciereszko, I., Staszak, A. M. (2019). Mitochondria are important determinants of the aging of seeds. International Journal of Molecular Sciences. 20(7):1568. https://doi.org/10.3390/ijms20071568

Sagisaka, S. (1976). The occurrence of peroxide in a perennial plant, Populus gelrica. Plant Physiology, 57:308-309.

Sako, K., Nguyen, M. H., & Seki M. (2020). Advances in chemical priming to enhance abiotic stress tolerance in plants. Plant and Cell Physiology, 61(12):1995-2003.

Sami, F., Faizan., M, Faraz, A., Siddiqui, H., Yusuf, M., & Hayat, S. (2018). Nitric oxide-mediated integrative alterations in plant metabolism to confer abiotic stress tolerance, NO crosstalk with phytohormones and NO-mediated post translational modifications in modulating diverse plant stress. Nitric Oxide, 73:22-38.

Sarath, G., & Mitchell, R.B., (2008). Aged switchgrass seed lot's response to dormancy-breaking chemicals. Seed Technology, 30(1):7-16.

Sen, A., Johnson, R., & Puthur, J.T. (2021). Seed priming: A Cost-effective Strategy to Impart Abiotic Stress Tolerance. In: Husen A. (ed.) Plant Performance Under Environmental Stress (Springer Nature Switzerland AG) 459-480.

Sepehri, A., & Rouhi, H. R. (2016). Enhancement of seed vigor performance in aged groundnut (Arachis hypogaea L.) seeds by sodium nitroprusside under drought stress. Philippine Agricultural Scientist, 99(4):339-347.

Singhal, R. K., Jatav, H. S., Aftab, T., Pandey, S., Mishra, U. N., Chauhan, J., Chand, S., Indu, Saha, D., Dadarwal, B. K., Chandra, K., Khan, M. A., Rajput, V. D., Minkina, T., Narayana, E. S., Sharma, M. K., & Ahmed, S. (2021). Roles of nitric oxide in conferring multiple abiotic stress tolerance in plants and crosstalk with other plant growth regulators. Journal of Plant Growth Regulation, 40:2303-2328.

Šírová, J., Sedlářová, M., Piterková, J., Luhová, L., & Petřivalský, M. (2011). The role of nitric oxide in the germination of plant seeds and pollen. Plant Science, 181(5):560-572.

Sougrakpam, Y., Babuta, P., & Deswal, R. (2018). Current Scenario of NO (S-Nitrosylation) Signaling in Cold Stress. In: Ramakrishna A., Gill S.S. (eds.) Metabolic Adaptations in Plants during Abiotic Stress (Taylor & Francis Group) 351-360.

Sougrakpam, Y., Babuta, P., & Deswal, R. (2023). Nitric oxide (NO) modulates low temperature-stress signaling via S-nitrosation, a NO PTM, inducing ethylene biosynthesis inhibition leading to enhanced post-harvest shelf-life of agricultural produce. Physiology and Molecular Biology of Plants, 29(12):2051-2065.

Turkan, I. (2017). Emerging roles for ROS and RNS—versatile molecules in plants. Journal of Experimental Botany, 68:4413-4416.

Venkatesan S., Masilamani P., Janaki P., Eevera T., Sundareswaran S., & Rajkumar P. (2020). Role of nitric oxide in seed biology and seed production: A review. Journal of Applied and Natural Science, 12(3):277-287.

Waqas M., Korres N.E., Khan M.D., Nizami Al.-S., Deeba F., Ali I., & Hussain H. (2019). Advances in the concept and methods of seed priming. In: Hasanuzzaman M., Fotopoulos V. (eds.) Priming and Pretreatment of Seeds and Seedlings (Springer Nature Singapore Pte Ltd.) 11-41.

Xia, F., Cheng, H., Chen, L., Zhu, H., Mao, P., Wang, M. (2020). Influence of exogenous ascorbic acid and glutathione priming on mitochondrial structural and functional systems to alleviate aging damage in oat seeds. BMC Plant Biology, 20(1): 104.

Yun, B. W., Feechan, A., Yin, M., Saidi, N. B., Le Bihan, T., Yu, M., Moore, J. W., Kang, J. G., Kwon, E., Spoel, S. H., Pallas, J. A., & Loake, G. J. (2011). S-nitrosylation of NADPH oxidase regulates cell death in plant immunity. Nature, 478(7368):264-268.

Zhang, K., Zhang, Y., Sun J., Meng J., Tao J. (2021). Deterioration of orthodox seeds during ageing: Influencing factors, physiological alterations and the role of reactive oxygen species. Plant Physiology and Biochemistry, 158:475-485.

Zhang, Y., Wang, L., Liu, Y., Zhang, Q., Wei, Q., Zhang, W. (2006). Nitric oxide enhances salt tolerance in maize seedlings through increasing activities of proton-pump and Na+/H+ antiport in the tonoplast. Planta, 224:545-555.