Acta Physiologiae Plantarum (2021) 43:117
https://doi.org/10.1007/s11738-021-03288-0

ORIGINAL ARTICLE

Gas-priming as a novel simple method of seed treatment with ethylene, hydrogen cyanide or nitric oxide

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Received: 24 January 2021 / Revised: 13 May 2021 / Accepted: 10 July 2021 / Published online: 17 July 2021

Abstract

The gases used: ethylene (C_2H_4), hydrogen cyanide (HCN) and nitric oxide (NO) showed a high activity as inductors of germination in primary dormant or non-dormant seeds exposed to stress or suboptimal temperatures. So far, research on the role of ethylene, hydrogen cyanide and nitric oxide has involved these gases during seed germination. This work describes gas-priming as a novel method for treating air dry seeds of the genus *Amaranthus* serving as a model. Effects of C_2H_4, HCN or NO applied to dry seeds were similar to those obtained when the gases were used during seed germination for an identical period of time. Application of the gases to air dry seeds presents a new opportunity to study the role of those gases in germination of dormant and non-dormant seeds and to constrain effects of the gases from time 0. The prolonged treatment time for dry seeds (24 h) is convenient because if the treatment is started in the morning, there is sufficient time the next day for further experiments to begin, e.g. germination test in water or in solutions of other compounds. Moreover, it is important that the gas-treated seeds can be stored or transported prior to use. The gas-priming method allows to prepare treated seed samples which can be used in experiments either immediately or after storage in open air or in a closed container. Gas-priming with C_2H_4, HCN and NO is a simple and useful treatment of air-dried seeds, which opens up new useful possibilities for basic research on the role of those gases in releasing dormancy and seed germination of various plant species. In addition, the method may prove very useful in horticulture and agriculture in improving germination of gas-sensitive seeds.

Keywords *Amaranthus* · Ethylene · Hydrogen cyanide · Nitric oxide · Priming · Seed germination

Abbreviations

C_2H_4	Ethylene
HCN	Hydrogen cyanide
NO	Nitric oxide

Introduction

Viable seeds of numerous species are not able to complete germination after harvest even when optimal germination conditions are applied. Such seeds are considered primarily dormant (Bewley 1997). Primary dormancy is particularly common in wild plants. Dormancy in seeds of both wild and domesticated plants can be removed by burial, stratification, dry storage or treatment with various chemicals, e.g. gibberellins, cytokinins, smoke and karrikins (Bewley et al. 2013; Kępczyński 2018). The balance between abscisic acid and gibberellins and/or sensitivity to these hormones are commonly thought to be mainly responsible for the establishment, maintenance and release of dormancy and for seed germination (Bewley et al. 2013). In addition to these compounds, ethylene and also other gaseous molecules, such as hydrogen cyanide (HCN) and nitric oxide (NO), are involved in regulation of seed dormancy.

Ethylene has been reported as an inductor of seed dormancy release (Corbineau and Come 1995; Kępczyński and Kępczyńska 1997; Matilla and Matilla-Vazguez 2008; Bogatek and Gniazdowska 2012; Arc et al. 2013) in many plant species, including the primarily dormant *Amaranthus retroflexus* (Kępczyński et al. 1996,2003,2017; Kępczyński and Sznigir 2013) and the secondarily dormant seeds of *Amaranthus caudatus* (Kępczyński et al. 2006). The hormone also stimulates *A. caudatus* seeds exposed to osmotic stress (Kępczyński and Karssen 1985; Kępczyński 1986) or inhibited by ABA (Kępczyński 1986) or methyl jasmonate

Communicated by M. Horbowicz.

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(Kępczyński and Białecka 1994). In all those studies on the role of ethylene in dormancy release and germination, seeds during imbibition on water were incubated in ethylene-enriched air or in the presence of a solution of ethephon, a compound releasing ethylene in plant tissues, or 1-aminocyclopropane-1-carboxylic acid (ACC), an ethylene biosynthesis precursor.

Gaseous molecules of HCN are highly volatile and soluble in water; the HCN water solution is known as hydrocyanic acid or prussic acid. The compound, very toxic to animals including humans, exerts a rapid paralyzing effect on most species; it can also cause injury in highly watery plant material, e.g. fruits. Because of its toxicity, HCN has been used so far as an effective fumigant against insects affecting seeds during dry storage. It also participates in regulation of various processes during plant development and serves an important function in seed dormancy release and germination (Siegień and Bogatek 2006; Flematti et al. 2013). The compound can be produced during ethylene biosynthesis as a co-product during the last reaction stage, ACC oxidation by ACC oxidase (ACO) (Peiser et al. 1984); thus, HCN is produced concurrently with ethylene synthesis. Moreover, HCN can be produced by catabolism of cyanide glucosides (Siegień and Bogatek 2006). HCN may act as a signaling molecule in regulation of processes in plants, including seed germination (Krasuska et al. 2015). Until now, the HCN treatment of seeds involved keeping the imbibed seeds in the atmosphere enriched with HCN released from KCN or Fe(II)CN (Bogatek et al. 1991; Bethke et al. 2007b; Sami et al. 2019, 2020).

The discovery of the gaseous molecule of NO, a highly reactive inorganic free radical performing an important function in animals stimulated research on its participation in regulating various processes in plant development, including releasing seed dormancy and germination. NO was found to be produced, via enzymatic and non-enzymatic pathways, not only in animal, but also in plant cells (Bethke et al. 2007a; Arc et al. 2013; Krasuska et al. 2015). The molecule is water-soluble and easily diffuses through cell membranes. The compound was found to play a crucial role in removing dormancy in seeds of several plants. NO was also found to stimulate germination of non-dormant seeds under optimal and stress conditions (Li et al. 2013). Effects of NO have been studied with various treatments. While the treatment employing purified gas has seldom been used, most experiments involved various donor compounds, e.g. sodium nitroprusside (SNP) *S*-nitroso-*N*-acetylpenicillamine (SNAP), *S*-nitrosoglutathione (GSNO) or acidified KNO_2, releasing NO to the atmosphere surrounding the seeds (Bethke et al. 2007a). In some experiments, seeds were imbibed in the donor (SNP) solution. At present, NO is recognized as a signaling molecule involved, alongside plant hormones, in regulating germination of dormant and non-dormant seeds (Arc et al. 2013; Krasuska et al. 2015). The importance of NO in dormancy release and germination is described by the "nitrosative door" model (Krasuska et al. 2015). According to the model, dormancy release requires reactive nitrogen species, mainly NO, at a suitable concentration. A too low concentration prevents germination, whereas a too high concentration leads to damage of cell components, thus inhibiting or delaying germination.

Air-dried seeds had been earlier treated with plant-derived smoke to induce germination (Kępczyński 2018), whereas HCN is used for treatment of air-dried seeds to protect them against insects during storage. So far, the role and mechanism of C_2H_4, HCN and NO in dormancy release and germination have been usually studied by treating the seeds with the gases mentioned during incubation in water. It was interesting to find out whether treating air-dried seeds with these gases would stimulate germination of dormant and non-dormant seeds.

This work was aimed at developing a new method for treating air-dried seeds with gases. The experiments were conducted with seeds of the genus *Amaranthus* as a model. To meet the objective of the work, it was important to find out whether (1) germination of non-dormant *Amaranthus caudatus* L. (an ornamental variety) seeds can be improved under osmotic stress or saline stress by C_2H_4 applied to air-dried seeds, (2) germination of non-dormant *Amaranthus cruentus* L. (a cultivated variety) seeds can be improved under osmotic stress or at suboptimal temperatures by HCN and NO applied to air-dried seeds, (3) dormancy of *Amaranthus retroflexus* L. (a weed) seeds can be removed by HCN or NO when the gases are applied to air-dried seeds. The effect of storing the C_2H_4-, HCN- and NO-treated dry seeds in open air or in a closed test tube on germination was determined as well. To compare effects of the gases applied with a more frequently used method, the seeds were also treated with the gases during germination.

Materials and methods

The experiments involved non-dormant seeds of *Amaranthus caudatus* L. cv. Atropurpureus, harvested in 2004 and *Amaranthus cruentus* L. cv. Aztek, harvested in 2006, both available commercially in Poland. Dormant seeds of *Amaranthus retroflexus* L. were harvested in 2009 from wild populations growing near Chojna in Poland. The inflorescences of the weed were dried at room temperature and gently shaken to remove the seeds. The air-dried seeds of all the species were stored at – 20 °C until used.

Seed treatments during germination

Amaranthus caudatus L. Three open Petri dishes (ø 6 cm) with seeds (50) on filter paper moistened with − 0.5 MPa PEG-8000 (1.5 ml) were placed in a tightly closed jar (0.5 l) to which C_2H_4 (ethylene) was injected with a syringe to obtain final concentrations of 10^{-7}, 5×10^{-7} and 10^{-6} M. Osmotic solutions of PEG-8000 were made up to Michel and Kaufman (1973). After incubation in darkness at 25 °C for 5 or 24 h, the Petri dishes with seeds were transferred to a 19-cm Petri dish (0.5 l) containing an open 6-cm with 5 ml water, and were kept up to 7 days in darkness at the same temperature. In one experiment, after 24 h incubation in the presence of − 0.5 MPa PEG and C_2H_4 at the same concentrations, the seeds were transferred to Petri dishes with a new PEG solution, and were incubated in air for up to 7 days. In another experiment, the seeds were incubated in Petri dishes in the presence of − 0.5 MPa PEG-8000 in a tightly closed jar (0.5 l) or in air enriched with ethylene, which was injected with a syringe to a tightly closed jar to obtain final concentrations of 10^{-7}, 5×10^{-7} and 10^{-6} M. Every day, the Petri dishes were taken out from jars, the germinated seeds were counted and, following airing, were returned to the jar with air or air enriched with ethylene to maintain the desired concentration.

Amaranthus retroflexus L. Three open Petri dishes (ø 6 cm) with seeds (50) on filter paper moistened with water (1.5 ml) were placed in a 19-cm Petri dish (0.5 l) containing an open 6-cm Petri dish with 5 ml water, 5 ml Fe(II)CN (10^{-4}, 2×10^{-2} M) or 5 ml KNO_2 (2×10^{-2}, 5×10^{-2} M) acidified with 5 ml 0.2 M HCl, and were sealed with three layers of Parafilm. After incubation for 1 day in light at 25 °C, the Petri dish with the donor solution was replaced with a Petri dish containing water. The 19-cm Petri dish, with smaller Petri dishes containing seeds and one with water, was kept at 25 °C in light for up to 7 days.

Treatment of air-dried seeds

Amaranthus caudatus L. Dry seeds were incubated for 24 h in a tightly closed jar (0.5 l) in dark at 25 °C in C_2H_4-enriched air to obtain concentrations of 5×10^{-7}, 10^{-6} or 1.5×10^{-6} M. The seeds were used immediately or after storage in open air for 24 or 48 h in a test tube sealed with three layers of Parafilm. Subsequently, the treated non-stored and stored seeds were transferred to three Petri dishes (50 seeds each) with filter paper moistened with 1.5 ml − 0.5 MPa PEG-8000 or 13.5×10^{-2} M NaCl and kept in a 19-cm Petri dish (0.5 l) containing an additional 6-cm Petri dish with water, in air at 25 °C in dark for up to 7 days.

Amaranthus cruentus L. Dry seeds were kept in a 19-cm Petri dish containing an open 6-cm Petri dish with 5 ml Fe(II)CN (10^{-3}, 3×10^{-3}, 2×10^{-2} M) or 5 ml KNO_2 (10^{-3}, 3×10^{-3}, 2×10^{-2} M) acidified with 5 ml 0.2 M HCl, sealed with 3 layers of Parafilm. After a 24 h treatment in light at 25 °C, the seeds were transferred to three Petri dishes with filter paper moistened with 1.5 ml − 0.5 MPa PEG-8000. The Petri dishes with seeds on the PEG solution were kept in a 19-cm Petri dish (0.5 l) containing an additional 6-cm Petri dish with water, in air at 20 °C in light or in air and in dark at 10 °C for 7 days.

Amaranthus retroflexus L. Dry seeds were placed in a 19-cm Petri dish containing an open 6-cm Petri dish with 5 ml Fe(II)CN (10^{-4}, 2×10^{-2} M) or 5 ml KNO_2 (2×10^{-2}, 5×10^{-2} M) acidified with 5 ml 0.2M HCl), sealed with three layers of Parafilm. After a 24 h incubation in light at 25 °C, the seeds were used immediately or after storage in open air for 24 h or in a test tube sealed with three layers of Parafilm for 48 h. The non-stored and stored treated seeds were transferred to three 6-cm Petri dishes with filter paper moistened with 1.5 ml water, and incubated in a 19-cm Petri dish containing an additional Petri dish with 5 ml water, in air and light at 25 °C for up to 7 days.

Seed germination

In some experiments, seed germination was checked every day during the 7 days of incubation, while in other tests only after 7 days. The seeds were regarded as germinated when the radicle protruded through the seed coat and was longer than ca. 2 mm.

Statistical analysis

All the experiments were carried out in triplicate; the results are expressed as mean ± SD. The means were tested for significance of differences ($P \leq 0.05$) using one- or two-way analysis of variance (ANOVA) and Duncan's multiple range test (Statistica for Windows v.12.0, Stat-Soft Inc., Tulsa, OK, USA).

Results

Effects of ethylene (C_2H_4) on germination of non-dormant *Amaranthus caudatus* seeds in the presence of PEG-8000

Application during germination

Non-dormant *Amaranthus caudatus* seeds germinated easily when incubated on water and in air (Fig. 1A). PEG-8000 at − 0.5 MPa markedly inhibited germination of seeds incubated in air; only 10–20% of the seeds were able to germinate. The seeds were incubated simultaneously in the presence of PEG at -0.5 MPa and in air enriched with

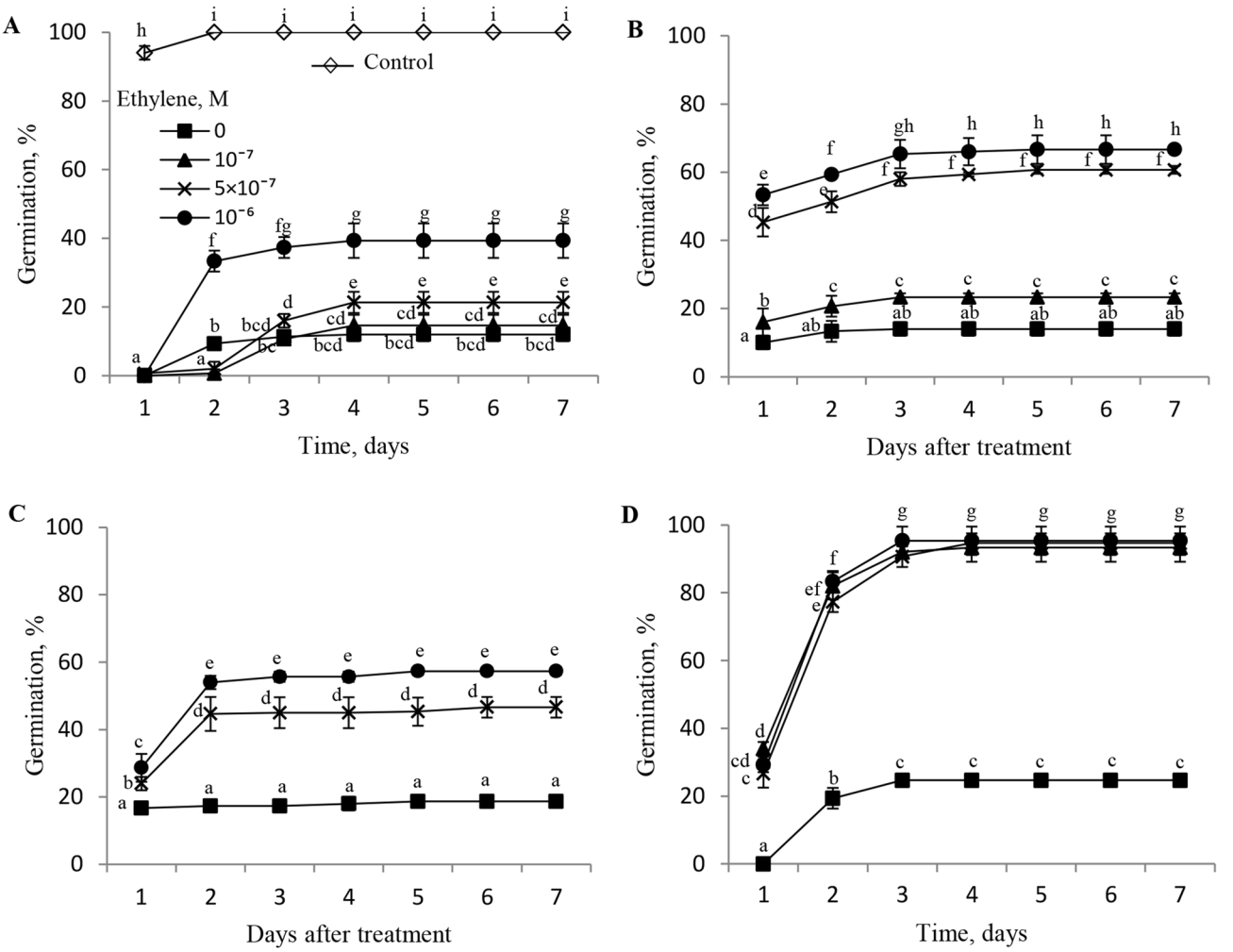

Fig. 1 Effects of C_2H_4, applied during incubation of *Amaranthus caudatus* seeds in the presence of − 0.5 MPa PEG for 5 h (**A**), 24 h (**B**, **C**) or 7 days (**D**) on germination. Vertical bars indicate ± SD. Two-way ANOVA with Duncan's post hoc test was used to test for significance of differences. Mean values with different letters are significantly different ($P < 0.05$). **A** Petri dishes with seeds on PEG solution after incubation for 5 h in air or in air enriched with ethylene were transferred to air. **B** Petri dishes with seeds on PEG solution after incubation for 24 h in air or in air enriched with ethylene were transferred to air. **C** Seeds were incubated for 24 h on PEG solution in air or in air enriched with ethylene were transferred to new PEG solution and incubated for 7 days in air. **D** Seeds were incubated on PEG solution for 7 days in air or in air enriched with ethylene; following daily airing, ethylene was added to maintain desired concentration

C_2H_4 at 10^{-7}, 5×10^{-7}, 10^{-6} M, for 5 (Fig. 1A) or 24 h (Fig. 1B); subsequently, the Petri dishes were transferred to air. C_2H_4 applied at the highest concentration (10^{-6} M) increased the germination percentage up to ca. 40% just after 2 days (Fig. 1A). When incubation was extended to 7 days, the germination rate was similar. The longer C_2H_4 treatment resulted in a higher level of germination (Fig. 1B). In addition, when applied at a lower concentration (5×10^{-7} M), C_2H_4 was able to stimulate germination. When the seeds were transferred to a new PEG solution after the 24 h C_2H_4 treatment, the stimulating effect of the compound used at 5×10^{-7}, 10^{-6} M was evident as well (Fig. 1C). The effect of the continuous presence of C_2H_4 on germination in PEG solution was also determined. The constant presence of C_2H_4 enabled germination of almost all the seeds even at the lowest C_2H_4 concentration (10^{-7} M) as soon as after 3 days (Fig. 1D).

Application to air-dried seeds

To examine a possibility of treating dry seeds with C_2H_4 to obtain a stimulatory effect, the dry seeds were incubated in air enriched with 5×10^{-7}, 10^{-6} and 1.5×10^{-6} M C_2H_4. The stimulating effect of C_2H_4 appeared when the treated dry seeds were sown onto the PEG solution immediately after treatment: 35% of the seeds germinated, 15%

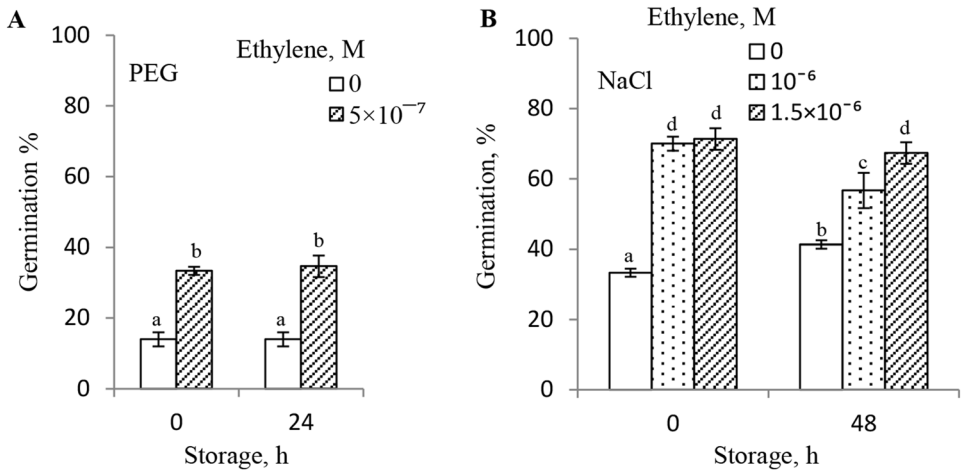

Fig. 2 Effects of C_2H_4, applied for 24 h to air-dried *Amaranthus caudatus* seeds, on germination in the presence of − 0.5 MPa PEG or 13.5 x 10^{-2} M NaCl after 7 days. Seeds were sown immediately after treatment, after storage in open air (**A**), or after storage in closed test tube (**B**). Vertical bars indicate ± SD. Two-way ANOVA with Duncan's post hoc test was used to test for significance of differences. Mean values with different letters are significantly different ($P < 0.05$)

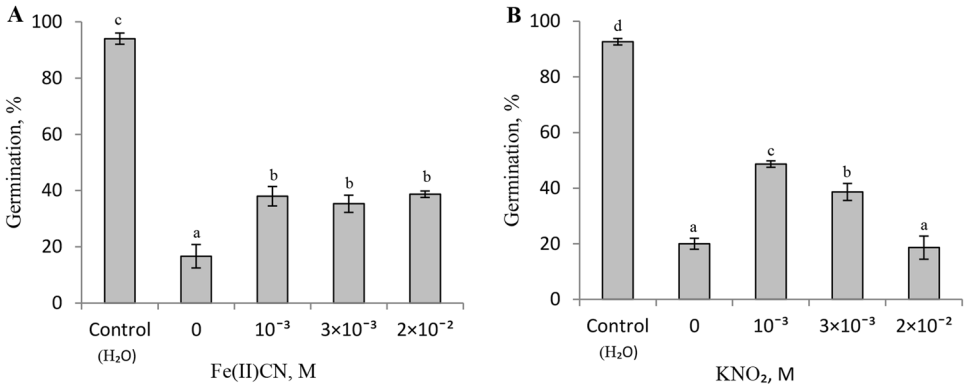

Fig. 3 Effects of HCN (**A**) and NO (**B**), applied for 24h to air-dried *Amaranthus cruentus* seeds, on germination in the presence of − 0.5 MPa PEG after 7 days. Vertical bars indicate ± SD. One-way ANOVA with Duncan's post hoc test was used to test for significance of differences. Mean values with different letters are significantly different ($P < 0.05$)

of the untreated seeds germinating in the presence of PEG (Fig. 2A). Storage of the treated seeds in open air for 24 h did not change the stimulating effect of C_2H_4. In another experiment, NaCl was used instead of PEG. NaCl at 13.5 x 10^{-2}M markedly inhibited germination: ca. 35% of the seeds germinated (Fig. 2B), whereas 100% germination was observed in water (Fig. 1A). Ca. 60–70% of the C_2H_4-treated dry seeds sown immediately germinated despite the presence of the salt solution. The stimulating effect of C_2H_4 also appeared after the treated seeds were stored for 48 h in a closed test tube.

Effects of hydrogen cyanide (HCN) and nitric oxide (NO) applied to air-dried non-dormant *Amaranthus cruentus* seeds on germination in the presence of PEG-8000 or at a suboptimal temperature (10 °C)

Non-dormant *A. cruentus* seeds incubated on water germinated almost completely; however, germination was markedly inhibited in the presence of -0.5 MPa PEG-8000, as ca. 20% of the seeds were able to germinate (Fig. 3). The HCN applied to dry seeds at concentrations of 10^{-3}, 3×10^{-3} and 2×10^{-2} M increased germination to ca. 40% (Fig. 3A).

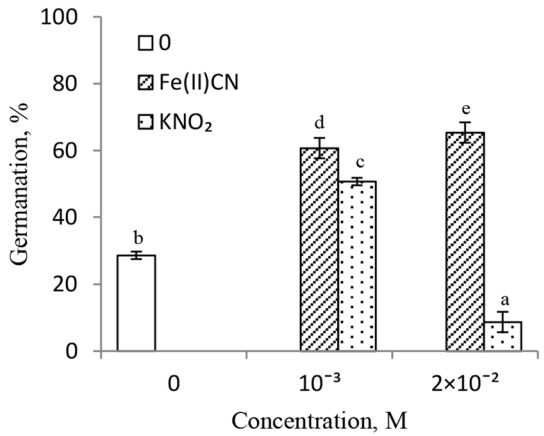

Fig. 4 Effects of HCN and NO, applied for 24h to air-dried *Amaranthus cruentus* seeds, on germination at 10 °C after 7 days. Vertical bars indicate ± SD. One-way ANOVA with Duncan's post hoc test was used to test for significance of differences. Mean values with different letters are significantly different ($P < 0.05$)

Dry seeds treated with NO at 10^{-3} or 3×10^{-3} M germinated better on the − 0.5 MPa PEG solution (ca. 40–50% germination) (Fig. 3B). Another experiment was conducted to

find out whether treating dry seeds with HCN or NO could improve germination at 10 °C, a temperature suboptimal for *A. cruentus* germination. At the temperature used, the seeds germinated poorly (30%) (Fig. 4). HCN at 10^{-3}, 2×10^{-2} M and NO at 10^{-3} M, applied to dry seeds, stimulated germination at the suboptimal temperature; ca. 50 and 60% of the seeds were able to germinate, respectively.

Effects of HCN and NO on germination of dormant *Amaranthus retroflexus* seeds

Application during germination

Dormant *A. retroflexus* seeds almost failed to germinate because of dormancy; as little as ca. 5–10% of the seeds could germinate (Fig. 5). HCN applied at 10^{-4} or 2×10^{-2} M during the 24 h incubation stimulated germination of the dormant seeds; the final germination percentage was ca. 30 or 40%, respectively. (Fig. 5A). The effect of 2×10^{-2} or 5×10^{-2} M NO used during the 24 h incubation on germination after transfer to air was tested as well. The germination was markedly enhanced, as 50–60% of the seeds germinated (Fig. 5B).

Application to air-dried seeds

HCN at 10^{-4} or 2×10^{-2} M was applied for 24 h to dry seeds which were sown immediately after the treatment. The germination percentage was higher (ca. 50%) than in the case of the untreated seeds (Fig. 6A). HCN was found to stimulate germination also when the treated seeds were stored for 24 h in open air. The 48 h storage of treated seeds in a closed tube did not seem to affect the stimulating effect of HCN. Seed germination was also determined after a 24 h treatment with 5×10^{-2} M NO. The germination percentage was observed to increase markedly when the seeds were sown directly after the treatment (ca. 75% of the seeds were able to germinate) (Fig. 6B). Storage of treated seeds in open air for 24 h or in closed tubes for 48 h only slightly reduced the stimulating effect of NO on germination.

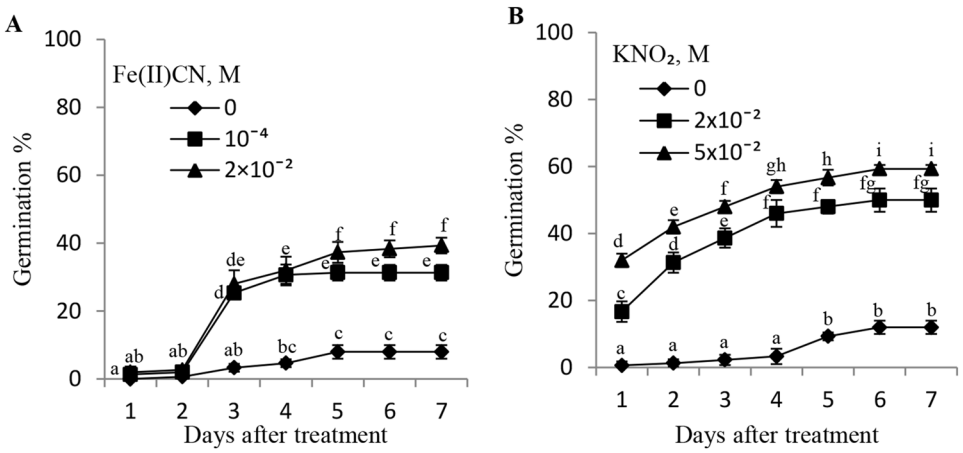

Fig. 5 Effects of HCN (**A**) and NO (**B**) applied to *Amaranthus retroflexus* seeds during 24 h incubation on water (**A**, **B**) on germination. Vertical bars indicate ± SD. Two-way ANOVA with Duncan's post hoc test was used to test for significance of differences. Mean values with different letters are significantly different ($P<0.05$)

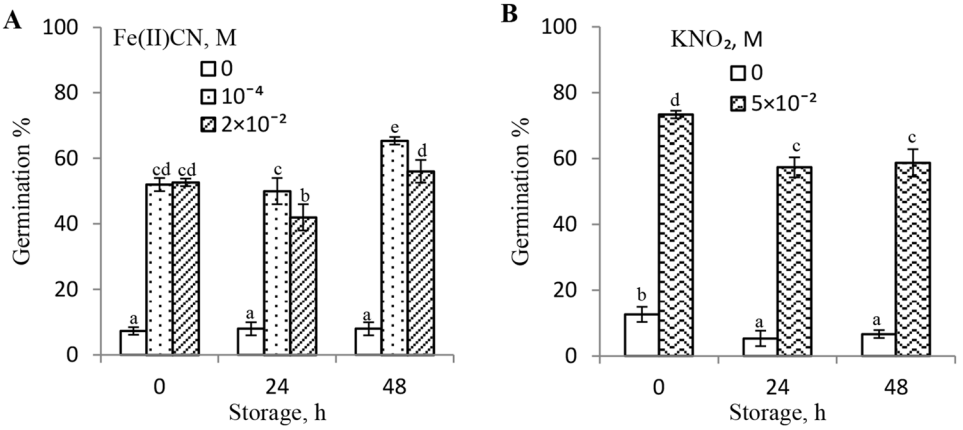

Fig. 6 Effects of HCN (**A**) and NO (**B**), applied for 24 h to air-dried *Amaranthus retroflexus* seeds, on germination after 7 days. Seeds were sown immediately after treatment, after storage in open air for 24 h or after storage for 48 h in closed test tube. Vertical bars indicate ± SD. Two-way ANOVA with Duncan's post hoc test was used to test for significance of differences. Mean values with different letters are significantly different ($P<0.05$)

Discussion

Ethylene

C_2H_4 is commonly accepted as a hormone very important for seed dormancy release and germination of various plant species (Kępczyński and Kępczyńska 1997; Matilla 2000). C_2H_4 also plays an important role in seed germination under non-optimal conditions. For example, the compound is able to alleviate the inhibitory effect of high temperature on germination of chickpea seeds (Gallardo et al. 1991) or the effect of salinity on halophyte seed germination (Khan et al. 2009). The results presented showed C_2H_4, applied for 24 h under osmotic stress, to improve *A. caudatus* seed germination to some extent when Petri dishes with seeds on the osmotic solution were transferred to air or when the seeds were transferred to a new solution and air (Fig. 1B, C). It had been shown earlier that both ethephon and C_2H_4 markedly reversed the PEG inhibition of *A. caudatus* seed germination (Kępczyński and Karssen 1985; Kępczyński 1986). The continuous presence of C_2H_4 during incubation of *A. caudatus* seeds in the osmotic solution completely alleviated the osmotic stress and all the seeds germinated, even at the lowest C_2H_4 concentration (Fig. 1D). Thus, the data presented indicate that C_2H_4 is required for longer than 24 h for the strongest effect to be obtained. Application of C_2H_4 to air-dried seeds for 24 h improved seed germination under osmotic stress as well (Fig. 2A), and the effect was comparable to that obtained when the compound was applied for 24 h simultaneously with osmotic stress (Fig. 1C). It turned out that C_2H_4-treated dry seeds can be stored for 24 or 48 h before use to alleviate osmotic or saline stress (Fig. 2A, B), so experiments do not have to be set up immediately after treatment.

Hydrogen cyanide

HCN was found to be a seed dormancy releasing factor in several plant species, e.g. *Oryza sativa* (Cohn and Hughes 1986), *Helianthus tuberosus* (Fol et al. 1989), *Malus domestica* (Bogatek et al. 1991). Like in previous studies, the compound markedly stimulated germination of dormant *Amaranthus retroflexus* seeds when applied during germination (Fig. 5A; Kępczyński and Sznigir 2014). When HCN was applied to air-dried seeds, the extent of stimulation was similar (Fig. 6A). HCN-treated seeds can be stored in open air for 24 h during which the compound's beneficial effect is not lost. HCN-treated dry seeds can be kept closed for 48 h and the stimulating effect of the gas would still be visible. HCN was also able to stimulate non-dormant *Amaranthus cruentus* seeds in osmotic solution when applied to dry seeds, thus indicating that osmotic stress can be alleviated (Fig. 3A). Moreover, germination of HCN-treated dry seeds of the species increased at the suboptimal temperature (Fig. 4). Thus, treating dry dormant *A. retroflexus* and non-dormant *A. cruentus* seeds with HCN is an effective method for releasing dormancy or improving germination under stress conditions or at a suboptimal temperature, respectively.

Nitric oxide

NO, released from various donors, was found to remove seed dormancy in several plants, e.g. *Arabidopsis thaliana*, *Hordeum vulgare*, *Malus domestica* (Bethke et al. 2007a, b; Krasuska et al. 2015). NO, applied during germination, can also release dormancy in *A. retroflexus* seeds (Liu et al. 2011; Kępczyński and Sznigir 2014; Kępczyński et al. 2017). The present study, too, showed NO applied during imbibition, to stimulate germination (Fig. 5B). As in the experiment with HCN, NO treatment of seeds produced a similar effect, regardless of how the seeds were treated. As with HCN, NO-treated air-dried seeds could be stored for 24 h in open air or for 48 h in closed tubes before testing, during which time the beneficial effect of NO would be maintained (Fig. 6B). NO applied to dry dormant *A. retroflexus* and non-dormant *A. cruenthus* seeds released dormancy and improved germination at osmotic stress or the suboptimal temperature (Figs. 3B, 4, 6B), respectively.

In summary, gas-priming is a new simple method of seed treatment. The effectiveness of applying gases to dry seeds for 24 h facilitates basic research, since there is a lot of time to set up experiments after a 1-day treatment. In addition, treating dry seeds is important in the study of the role of individual gases in dormancy and seed germination, because the impact of gases can be followed from the moment of contact with water. Similarly, from the standpoint of a potential practical application, the availability of treated seeds early on the next day gives ample time for sowing in Petri dishes or in the field. Undoubtedly, a possibility of storing the treated seeds in open air or in a closed container before an experiment can be set up is very advantageous. It is worth emphasizing that the advantage of gas-priming lies in its allowing to store treated seeds, which facilitates transporting the seeds to another laboratory or possibly to a site of planned cultivation.

Author contribution statement JK conceived and designed the research, interpreted the results, wrote the manuscript.

Acknowledgements I would like to express our gratitude to Prof. Ewa Kępczyńska for reading and commenting on the manuscript. I wish to thank Kinga Rybak, M.Sc. for technical assistance. I am indebted to Dr Teresa Radziejewska for linguistic assistance.

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