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Nitric Oxide as Germination Controlling Factor in Seeds of Various Plant Species

By

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With 1 Figure

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Summary

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Nitric oxide (NO) is an inorganic free radical, that plays an important role in regulation of a variety processes in plants. This small gaseous molecule is known as dormancy removing factor during seed germination of various plant species and probably plays role in activation of embryonic axes. The influence of sodium nitroprusside (SNP) a commonly used NO donor on seed germination of weed species: pigweed (*Amaranthus retroflexus*) and goosefoot (*Chenopodium album*) and crops: sugar beet (*Beta vulgaris*), barley (*Hordeum vulgare*) and Chinese cabbage (*Brassica pekinensis*) was investigated. SNP stimulated germination of sugar beet, barley and pigweed seeds in dose dependent manner. Additionally, in seedlings of sugar beet developed from seeds treated with SNP the improvement of root and shoot growth was detected. The obtained results suggest that SNP maybe a useful chemical in seed priming, leading to acceleration of seedling growth and development.

Zusammenfassung

Gniazdowska A., Babańczyk T. & Krasuska U. 2012. Nitric oxide as germination controlling factor in seeds of various plant species. [Stickstoffmonoxid als Fak-

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tor, der Samenkeimung von verschiedenen Pflanzenarten kontrolliert]. – Phyton (Horn, Austria) 52 (2): 219–226, mit 1 Abbildung.

Das Stickstoffmonoxid (NO) ist ein freies Radikal, das eine wesentliche Rolle in der Regulation von vielen physiologischen Prozessen bei Pflanzen spielt. Dieses kleine Gasmolekül gilt als Faktor, der die Keimruhe von vielen Pflanzengattungen aufhebt. NO wird emittiert während der frühen Keimphasen und nimmt an der Aktivierung der Keimlingsachse teil. In der Untersuchung wurde der Einfluss von Nitroprussid-Natrium (NPN), des allgemein angewendeten Spenders von NO, auf Samenkeimung von Unkräutern wie Amarant (*Amaranthus retroflexus*) und Weissem Gänsefuss (*Chenopodium album*) sowie Nutzpflanzen: Zuckerrübe (*Beta vulgaris*), Chinakohl (*Brassica pekinensis*) und Gerste (*Hordeum vulgare*) untersucht. Es wurde die Stimulierung der Samenkeimung von Zuckerrübe, Gerste und Weissem Gänsefuss beobachtet, wobei die Wirksamkeit von NPN von der Konzentration abhängig war. Darüber hinaus wurde für Zuckerrüben-Sämlinge, die von den mit NPN behandelten Samen herangewachsen sind, eine verbesserte Entwicklung von Wurzeln und Sprossen beobachtet. Die Ergebnisse der Untersuchungen zeigen, dass NPN für die Qualitätsverbesserung vom Saatmaterial durch die Anwendung von diesem Reagens während des Prozesses von Vorkonditionierung der Keime angewendet werden kann. Im Endergebnis kann die Behandlung der Keime mit NPN zur Beschleunigung vom Keimwachstum und -entwicklung führen.

Introduction

Soils are an important source of nitric oxide (NO), an inorganic free radical, that plays an significant role in regulation of variety processes in plants e.g. senescence, ripening, root growth or gravitropic bending (Crawford & Guo 2005). It is suggested that NO may be a signaling molecule acting as seed dormancy breaking agent and stimulator of germination (for review see Giba & al. 2006, Bethke & al. 2007b). Enhancement of seeds germination after their treatment with different donors of NO was demonstrated for various plant species e.g. *Arabidopsis thaliana* (Batak & al. 2002, Bethke & al. 2006), flixweed (*Descurainia sophia*), *Suaeda salsa* (Li & al. 2005), lettuce (*Lactuca sativa*) (Beligni & Lamattina 2000), yellow lupine (*Lupinus luteus*) (Kopyra & Gwóźdź 2003), switchgrass (*Panicum virgatum*) (Sarath & al. 2006), common wheat (*Triticum aestivum*) (Zhang & al. 2005). In our previously published papers we demonstrated that NO applied as SNP, SNAP or acidified nitrite terminate deep embryonic dormancy of apple (*Malus domestica*) embryos and accelerated their germination and development of young seedlings (Gniazdowska & al. 2010a). Moreover, NO mode of action during seed germination involves interaction with classical phytohormones: ethylene and abscisic acid (ABA) (Gniazdowska & al. 2007, 2010b). In *A. thaliana* seeds a close relation of NO to gibberellins was also proved (Bethke & al. 2007a). SNP is the cheapest and most commonly used donor of NO. During its decomposition on light NO^+ is emitted together with CN^-. Cyanide, similarly to NO although toxic in higher concentration, may act as stimulator of many processes in plants,

including seed germination (Siegień & Bogatek 2006). Therefore it is possible that SNP may be used to improve seed quality when applied during seed priming.

The aim of our work was to investigate the effect of SNP on germination of seeds of various plant species. We tested seed of weed plants such as pigweed (*Amaranthus retroflexus*) and goosefoot (*Chenopodium album*) and several crops: sugar beet (*Beta vulgaris*), barley (*Hordeum vulgare*) and Chinese cabbage (*Brassica pekinensis*).

Material and Methods

Plant Material

The experiments were carried out on seeds of 3 crop plant: sugar beet (*Beta vulgaris* sub. *vulgaris* convar. *crassa* provar. *altissima*), barley (*Hordeum vulgare*), Chinese cabbage (*Brassica pekinensis*) and 2 common European weeds: pigweed (*Amaranthus retroflexus*) and goosefoot (*Chenopodium album*).

Germination Tests

Seeds (25–50 depending on their size) were germinated on Petri dishes (∅ 9 cm) filed with filter paper wetted with 5 ml distilled water (control) or water solution of sodium nitroprusside (SNP) at various concentration 0.025–5.0 mM. Pigweed and barley were treated with 0.05, 0.1 and 0.2 mM SNP, goosefoot and Chinese cabbage with 0.025, 0.05, 0.1 and 0.2 mM SNP, while sugar beet with 0.125, 0.25, 0.5, 1.0, 2.5 and 5.0 mM SNP. SNP solution was prepared immediately before the experiment. Seeds were cultured in a Versalite Environmental Test chamber Sanyo. Sugar beet, barley and pigweed were germinated in 12-h photoperiod in temperature 25 °C/20 °C day/night, goosefoot and Chinese cabbage were germinated in 12 h photoperiod, temperature 21 °C/15 °C day/night, quantum light was 150 μmol PAR m^{-2} s^{-1}, humidity 60%.

For sugar beet seeds additional experiment was performed. Dry sugar beet seeds were imbibed in distilled water for 12 h and then transferred to Petri dishes filled with SNP solution at various concentration. The prolonged culture was continued as described above.

Seed germination was counted for 4–11 days depending on the plant species (11 days for sugar beet, 4 days – Chinese cabbage, 3 days – barley, 9 days for pigweed and 6 days for goosefoot). Seeds were considered to have germinated when radicles were 2–3 mm long.

Statistical Analysis

The data are based on three to five sets of experiments with the assay conducted in triplicate. Data were analyzed using the StatGraphics 5.1 Software. Mean values were computed for each experiment and mean differences were calculated using Tukey's studentized range test.

Results and Discussion

SNP is frequently used as one of NO donor. This chemical is easy diluted in water and simple in application. Due to its low price it is possible

to arrange experiment with SNP on a large scale, or even to apply it commercially. We demonstrated that SNP at millimolar concentration enhanced seed germination of tested crop species, while usually inhibited germination of seeds of weed species.

Germination of goosefoot (*Chenopodium*) seeds was stimulated by SNP applied only in lowest concentration. SNP 0.025 mM accelerated germination of goosefoot seeds and enhanced in 13 % seed germination 6 days after sowing. SNP in higher concentration 0.05 mM and above decreased goosefoot seed germination (Table 1).

Table 1. Germination rate (%) of goosefoot (*Chenopodium album*) seeds in water (control) or in SNP water solution at various concentration (0.025, 0.05, 0.1 and 0.2 mM) determined 2, 4 and 6 days after sowing.

	SNP mM				
days	control	0.025	0.05	0.1	0.2
2	20 ± 2	25 ± 2*	12 ± 1*	13 ± 2*	14 ± 1*
4	43 ± 4	47 ± 5	33 ± 4*	36 ± 6	27 ± 3*
6	62 ± 5	75 ± 5	42 ± 3*	48 ± 4*	33 ± 4*

* ($P < 0.05$). Treatments differ significantly from the control.

Pigweed (*Amaranthus retroflexus*) seeds germinated well in 0.05 mM SNP, but an evident stimulation of germination was detected only at the beginning of culture, at the stage between 2–5 days after sowing (Table 2). SNP water solution at higher concentration (0.2 mM) delayed pigweed seeds germination at the beginning of culture, but final germination measured 9 days after sowing was only 7 % lower than in control seeds (Table 2).

Table 2. Germination rate (%) of pigweed (*Amaranthus retroflexus*) seeds in water (control) or in SNP water solution at various concentration 0.05, 0.1 and 0.2 mM counted 2, 3, 5, 7, 9 days after sowing.

	SNP mM			
days	control	0.05	0.1	0.2
2	9 ± 1	29 ± 2*	16 ± 2*	5 ± 1*
3	32 ± 3	52 ± 4*	39 ± 3	6 ± 1*
5	43 ± 3	55 ± 4*	68 ± 4*	53 ± 3*
7	64 ± 4	61 ± 4	72 ± 6*	67 ± 5
9	85 ± 5	81 ± 6	75 ± 6	78 ± 6

* ($P < 0.05$). Treatments differ significantly from the control.

In literature, there are only rare data on the impact of SNP on germination of weed species. Promotion of seed germination by SNP was detected for flixweed (*Descurainia sophia*) abundant in North America and China (Li & al. 2005). SNP (0.1–0.4 mM) stimulated flixweed seed germination in concentration dependent manner.

One of the first data on SNP function in plant physiology was an observation done on lettuce seeds (BELIGNI & LAMATTINA 2000). SNP at concentration 0.1 mM led to almost 100% germination of lettuce seeds, while in 0.01 mM SNP only 50 % of seeds germinated. In our experiments we used Chinese cabbage, sugar beet and barley seeds. Germination of seeds of Chinese cabbage and barley seems to be less sensitive to SNP in low concentration than germination of seeds of two tested weed species. Germination of barley seeds as well as Chinese cabbage was only slightly influenced by SNP (Table 3 and 4). Both barley and Chinese cabbage seeds germination was stimulated mostly by 0.1 mM SNP. Moreover germination of barley seeds was slightly inhibited in 0.2 mM SNP, while SNP at such concentration did not reduce germination of Chinese cabbage seeds (Table 4). In water seeds of Chinese cabbage (control) germinated after 2 or 3 days in 72 and 83 % respectively. 2 days after sowing about 15 % stimulation of seed germination was detected after treatment with 0.1 mM SNP, while 3 days after sowing almost 100% of SNP (1 mM) treated seeds germinated (Table 4). SEN 2010 reported that SNP at low (0.1 mM) concentration did not influenced germination, while at highly elevated (10 mM) concentration inhibited germination of wheat seeds. In addition BETHKE & al. 2004 observed a stimulatory effect of SNP (0.1–0.5 mM) on dormancy removal of three cultivars of malting barley. Moreover the involvement of SNP into regulation of seed germination was proved on model plant. BETHKE & al. 2004, 2006 demonstrated for several times that seed germination of *A. thaliana* may be influenced by treatment with SNP or other NO source.

Table 3. Germination rate (%) of barley (*Hordeum vulgare*) seeds in water (control) or in SNP water solution at various concentration 0.05, 0.1, 0.2 mM calculated 2 and 3 days after sowing.

	SNP mM			
days	control	0.05	0.1	0.2
2	50 ± 4	52 ± 4	78 ± 6*	45 ± 5
3	70 ± 3	68 ± 3	98 ± 2*	63 ± 5

* ($P < 0.05$). Treatments differ significantly from the control.

Table 4. Germination rate (%) of Chinese cabbage (*Brassica pekinensis*) seeds in water (control) or in SNP water solution at various concentration 0.025, 0.05, 0.1, 0.2 mM determined 2 and 3 days after sowing.

	SNP mM				
days	control	0.025	0.05	0.1	0.2
2	72 ± 3	82 ± 6	86 ± 4*	87 ± 5*	83 ± 4
3	83 ± 2	92 ± 4	97 ± 3*	98 ± 2*	90 ± 5

* ($P < 0.05$). Treatments differ significantly from the control.

Table 5. Germination rate (%) of sugar beet seeds in water (control) or in SNP water solution at various concentration 0.125, 0.25, 0.5, 1.0 mM 4,6,7 and 11 days after sowing.

	SNP mM						
days	control	0.125	0.25	0.5	1.0	2.5	5.0
4	21 ± 2	11 ± 2*	17 ± 2	26 ± 3	11 ± 4*	9 ± 4*	14 ± 2
5	26 ± 4	20 ± 3	24 ± 4	44 ± 5*	19 ± 3	17 ± 3	20 ± 3
6	32 ± 3	29 ± 3	32 ± 4	48 ± 5*	29 ± 4	28 ± 4	33 ± 2
7	45 ± 4	39 ± 5	50 ± 4	60 ± 6*	39 ± 3	40 ± 5	48 ± 4
11	49 ± 2	65 ± 3*	71 ± 2*	79 ± 5*	51 ± 4	48 ± 5	51 ± 6

* ($P < 0.05$). Treatments differ significantly from the control.

Table 6. The influence of 12 h pre-imbibition in water on germination of sugar beet seeds after treatment with SNP water solution. Seed germination in SNP solution after pre- imbibitions in water was expressed in % of seed germination without pre-imbibition.

	SNP mM	
days	0.25	0.50
4	190 ± 18	93 ± 12
7	97 ± 10	80 ± 8
11	92 ± 8	85 ± 15

In our research, the most interesting results were obtained on sugar beet seeds (Table 5). In water after 11 days germinated only 50 % of sugar beet seeds. SNP at higher (1.0, 2.5 and 5.0 mM) concentration had no beneficial nor inhibiting effect on seed germination. While 0.25 and 0.5 mM SNP significantly enhanced germination to 71% and 79 % respectively after 11 days. Final germination of seeds treated with SNP at 0.125 mM concentration calculated 11 days after sowing was about 30 % higher than in control. Additionally, it should be noted that 0.5 mM SNP accelerated germination of sugar beet seeds. Five days after sowing almost twice more seeds treated with 0.5 SNP germinated in comparison to the control. Pre-imbibition of sugar beet seeds in water for 12 h stimulated germination of seeds treated with only 0.25 mM SNP and determined 4 days after sowing (Table 6). In other treatments pre-imbibition of seeds in water did not influence their final germination. Although SNP impact on sugar beet seeds germination was not so impressive it should be underlined that SNP visibly stimulated growth and development of young seedlings (Fig. 1). Similar acceleration in development of young apple seedlings was observed also in our previous experiments (Gniazdowska & al. 2010a). Moreover we demonstrated that even short term SNP treatment of young apple seedlings with morphological anomalies led to removal of abnormalities, resulting in growth of typically developed plants. Therefore, it is possibly that acceleration in growth of sugar beet seedling by SNP may finally lead

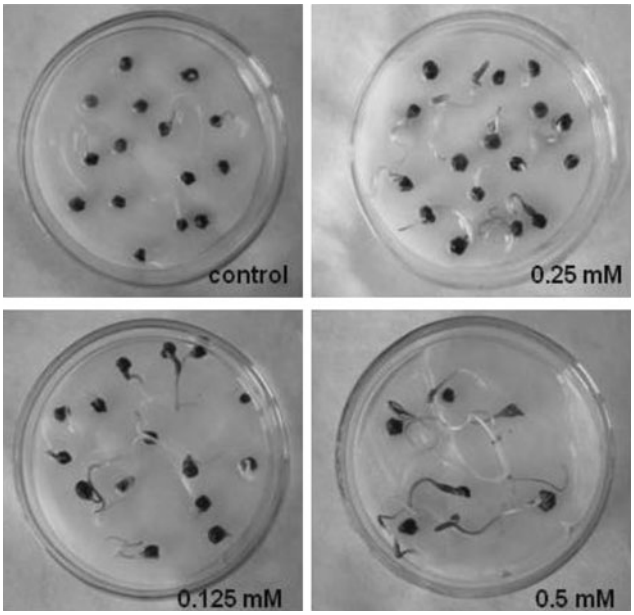

Fig. 1. Seedlings (7 days old) developed from control sugar beet seeds germinated in water and seedlings developed from sugar beet seeds treated with SNP at different concentration (0.125, 0.25, 0.5 mM).

to enhancement yielding of this crops. Depending on the presented results we suggest that SNP might be used for seed priming, mainly for sugar beet seeds. It may lead to acceleration of seedling growth and development. In future, some field experiments are necessary to verify this proposal since involvement of various environmental stresses would influence beneficial effect of SNP on seed germination.

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