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RAPID COMMUNICATION

Breaking the apple embryo dormancy by nitric oxide involves the stimulation of ethylene production

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Abstract Mature seeds of apple (*Mallus domestica* Borb. cv. Antonówka) are dormant and do not germinate unless their dormancy is removed by several weeks of moist-cold treatment. We investigated the effect of short-term (3 h) nitric oxide (NO) pretreatment on breaking of apple embryonic dormancy expressed as inhibition of germination and morphological abnormalities of young seedlings. Imbibition of embryos isolated from dormant apple seeds with sodium nitroprusside (SNP) or *S*-nitroso,*N*-acetyl penicillamine (SNAP) as NO donors resulted in enhanced germination. Moreover, NO treatment removed morphological abnormalities of seedlings developing from dormant embryo. The NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-teramethylimidazoline-1-oxyl-3 oxide (cPTIO) removed the above effects. NO-mediated breaking of embryonic dormancy correlated well with enhanced ethylene production. Inhibitor of ethylene synthesis (AOA) reversed the stimulatory effect of NO donors on embryo germination. Additionally SNP reduced embryo sensitivity to exogenously applied ABA ensuing dormancy breakage. We can conclude that NO acts as a regulatory factor included in the control of apple embryonic dormancy breakage by stimulation of ethylene biosynthesis.

Keywords Apple embryo · Ethylene · Nitric-oxide donors · Seed dormancy · Seed germination

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Abbreviations

ABA	Abscisic acid
ACC	1-aminocyclopropane-1-carboxylic acid
AOA	Aminooxyacetic acid
cPTIO	2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3 oxide
GA	Gibberellic acid
SNP	Sodium nitroprusside
NO	Nitric oxide
SNAP	*S*-nitroso,*N*-acetyl penicillamine

Introduction

The dormancy in apple seeds is well expressed, its embryonic dormancy is completely removed by several weeks of cold stratification. The physiological, morphological and metabolic aspects of apple embryo dormancy and its consequences for seedling development were investigated in details in the past (Bogatek 1995; Bogatek et al. 1991, 2002). Apple embryos isolated from dormant seeds germinate but the germination is markedly slower than that of stratified seeds and seedlings developing from such embryos are characterised by morphological anomalies. Our previous observations indicated that short pretreatment with HCN (6 h, 1 mM) of apple embryos leads to dormancy breakage and an increase in germination rate. Moreover, HCN pretreatment eliminates all morphological abnormalities observed in seedlings developing from dormant embryos, e.g., asymmetric growth and greening of cotyledons, inhibition of hypocotyls and internode elongation growth (Bogatek et al. 1991). The similar to HCN stimulation of germination by nitric oxide (NO) was detected on other seeds (lettuce, *Arabidopsis* and

barley) (Beligni and Lamattina 2000; Bethke et al. 2004, 2006), although those seeds are not characterized by deep dormancy.

Nitric oxide plays a role in the control of plant growth and development, fruit ripening, senescence, stomata movement and pathogen defense (Lamattina et al. 2003; del Rio et al. 2004). The cross talk of NO with classical hormones as auxins, cytokinin, gibberellins and ABA was also discussed (Leshem et al. 1998; Scherer 2000; Neill et al. 2002; Pagnussat et al. 2002; Hung and Kao 2003; Correa-Aragunde et al. 2006). Interaction between NO and ethylene were studied only in connection with fruit maturation and leaves senescence processes (Leshem et al. 1998), but there are no data concerning relation between NO dependent stimulation of germination and ethylene production in seeds. A certain correlation was found between the concentration of endogenous ethylene in seeds and their germinability. Moreover, the exogenous ethylene accelerated the germination of many dormant and non-dormant seeds and isolated embryos (Kępczyński and Kępczyńska 1997 and references herein; Calvo et al. 2004).

The aim of our study was to examine interaction between NO-dependent dormancy breakage, seedling development and ethylene production in apple embryos.

Materials and methods

Plant material

The experiments were carried out on apple (*Malus domestica* Borb., cv. Antonówka) seeds harvested in 2005. Seeds containing ca. 10% water were stored in sealed containers at 5°C. Seed coat and endosperm were removed from seeds imbibed for 24 h in distilled water at 18–20°C. The naked embryos were taken for determination.

Seed germination

Naked apple embryos isolated from dormant seeds were exposed to NO donors—sodium nitroprusside (SNP) or *S*-nitroso,*N*-acetyl penicillamine (SNAP) at a concentration of 5 mM for 3 h in the light. Then, the embryos were rinsed in distilled water and transferred to Petri dishes containing filter paper wetted with water. Control (untreated) and NO-pretreated embryos were germinated on Petri dishes (30 embryos per dish) at 20°C with 12/12 h (light/dark) photoperiod, under 150 μmol PAR $m^{-2} s^{-1}$. To check the specificity of NO action during germination of apple embryos light inactivated SNP or 0.3 mM 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3 oxide (cPTIO; NO scavenger) were used. Light inactivated SNP was prepared by exposition of SNP water solution to direct sunlight for 1 day or to artificial light for 2–3 days as described by Seregelyes et al. (2003). Such treatment results in complete conversion of SNP to sodium prusside.

For experiments with abscisic acid (ABA), control and SNP-pretreated embryos were transferred to Petri dishes containing filter paper wetted with ABA at different concentration (0.1, 0.3, 1, 3 μM).

For experiments with inhibitor of ethylene biosynthesis, control and SNP (5 mM, 3 h) pretreated embryos were transferred to Petri dishes containing filter paper wetted with 1 mM aminooxyacetic acid (AOA) and germinated as described above.

Germination tests were also done using control (dormant) embryos imbibed in 1 mM ethrel (ethylene donor). To verify the specificity of the ethrel effects embryos were imbibed with 2 mM phosphoric acid in 50 mM phosphate buffer (pH 7.0) or 50 mM phosphate buffer (pH 7.0) as described by Calvo et al. (2004). In additional experiments embryos were exposed to ethrel vapors. Uncovered embryos-containing Perti dish was placed in 500 ml glass flask with a dish containing 5 ml ethrel (2 mM) solution. After 2 days embryos were transferred to fresh distilled water. All these treatments were performed in growth chamber. Embryos were judged to have germinated when radicle was 2–3 mm long. All germination tests were repeated 3–5 times and presented data correspond to means ± SD.

Ethylene production

Dormant, SNP-, SNAP-, and cPTIO-pretreated embryos or young seedlings were used in the experiments to determine ethylene production. Plant material was collected and incubated in airtight 10 ml glass flasks for 2 h at 30°C to measure ethylene evolution. Ethylene content in the gas phase was detected using an Agilent 6890 gas chromatograph (Hewlett Packard, USA) equipped with a flame ionization detector and a stainless-steel column (3 m long, 3.5 mm inner diameter) packed with 80/100 Poropack Q.

Chemicals

cPTIO (Sigma) was made to 50 mM stock in distilled H_2O and stored at −18°C. ABA (Sigma) was made to a 100 μM stock solution with distilled H_2O and stored at

−18°C; 5 mM SNP and 5 mM SNAP as well as 1 mM AOA (Sigma) water solutions were prepared immediately before use.

Results

Nitric-oxide donors reduce dormancy of apple embryos and remove morphological abnormalities of the seedlings

Embryos isolated from dormant apple seeds germinate slowly and after 8 days of imbibition only 16% of them germinated. Sodium nitroprusside, a commonly used NO donor reduced dormancy of apple embryos imbibed in the light (Figs. 1, 2). Apple embryos' continuous imbibition in SNP (0.05–5 mM) resulted in stimulation of germination and this effect was dose dependent, although after 6 days SNP at highest concentration caused chlorophyll degradation and visible symptoms of embryos death (data not shown). Stimulation of germination was more pronounced for embryos shortly (3 h) pretreated with SNP at high concentration (1, 2.5 and 5 mM) (data not shown). The best effects were obtained with 5 mM SNP (3 h) pretreatment, promoting embryos germination in almost 60% after 8 days (Fig. 1). Seedlings obtained from SNP (5 mM, 3 h) pretreated embryos do not demonstrate any morphological abnormalities (Fig. 2) typical for seedlings developing from dormant embryos. Visible elongation of radicle and its gravitropic curvature began between 4 and 5 days of imbibition and was followed by increase in size of cotyledons and it is greening. Both cotyledons of SNP pretreated embryos were growing symmetrically and most of them were completely green (Fig. 2). The effect of SNP was reversed by NO scavenger cPTIO, after 8 days of imbibition only 25% of embryos germinated. cPTIO strongly maintained dormancy of dormant (untreated) embryos, after 8 days only 5% of embryos germinated (Fig. 1). Additional experiments with SNAP were conducted to show the effect of NO on breaking embryonic dormancy, since it was reported that SNP may act differentially from other NO donors (Murgia et al. 2004). We compared the effect of short time SNP (5 mM, 3 h) and SNAP (5 mM, 3 h) pretreatment on apple embryos dormancy breakage (Fig. 1). Pretreatment (3 h) of dormant apple embryos with 5 mM SNAP resulted in stimulation of germination, and after 8 days almost 50% of embryos germinated (Fig. 1). Imbibition with cPTIO (0.3 mM) after SNAP short-term pretreatment reversed stimulation of germination of apple embryos (Fig. 1). Additionally after 8 days of imbibition with light inactivated SNP solution only 22% of embryos germinated (data not shown). Since the final extent of germination induced by SNP and SNAP were similar, other germination tests were done on embryos pretreated with 5 mM SNP for 3 h.

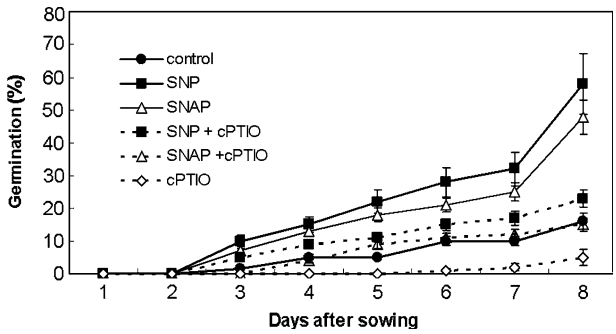

Fig. 1 Germination time courses for control (dormant) apple embryos, control embryos imbibed in cPTIO, embryos pretreated with SNP (5 mM, 3 h) or SNAP (5 mM, 3 h) and then imbibed in water, or embryos imbibed in cPTIO after SNP or SNAP (5 mM, 3 h) pretreatment. Data points at each time are means ± SD of five replicates

Nitric-oxide donors increase ethylene production by apple embryos

Ethylene emission was determined after embryos pretreatment by SNP, SNAP and cPTIO. Ethylene emission by embryos exposed to 5 mM SNP or SNAP for 3 h was detected just after 3 days of germination, whereas in control embryos it was noticed 24 h later. In the prolonged culture it increased rapidly in embryos pretreated by NO-donors, reaching two fold higher value as compared to the control after 6 days of germination (Fig. 3). Ethylene emission by SNAP-pretreated embryos was approximately 20% lower as compared to ethylene emission by SNP pretreated embryos. cPTIO reversed stimulation of ethylene production by SNAP pretreated embryos (Fig. 3).

When dormant embryos were imbibed in ethrel-donor of exogenous ethylene approximately 53% of embryos germinated after 8 days (Fig. 4), reaching the final value similar to that observed after SNP or SNAP pretreatment. Germination of apple embryos imbibed with 2 mM phosphoric acid in 50 mM phosphate buffer (pH 7.0) or 50 mM phosphate buffer was similar to dormant ones. Only 13 or 17% embryos, respectively, germinated after 8 days (data not shown). It was reported previously that during decomposition of ethrel, ethylene is the only gas evolved (Biddle et al. 1976). Therefore we checked

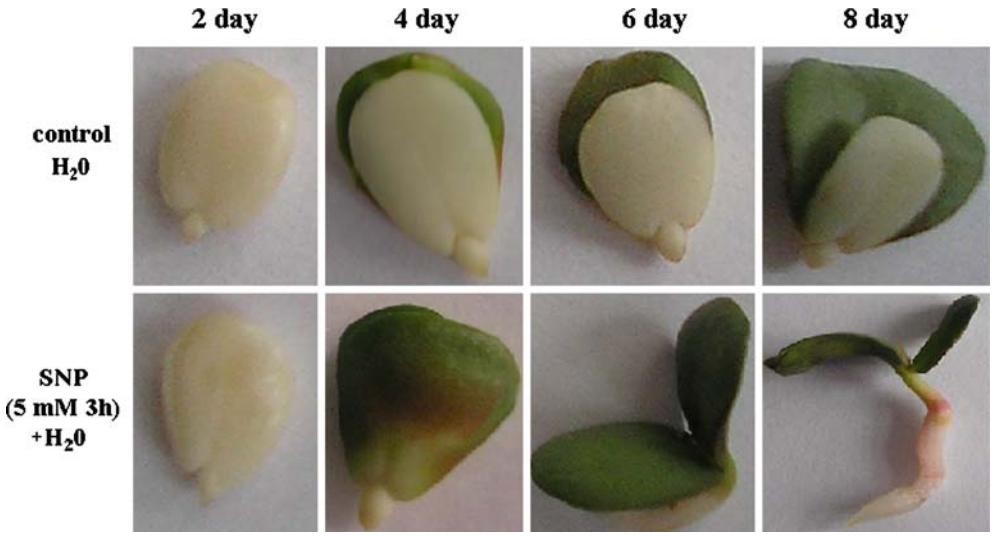

Fig. 2 NO-donor (SNP) removes embryonic dormancy of apple. SNP pretreatment (5 mM, 3 h) enhanced germination on apple embryos and stimulates synchronic growth of the seedling

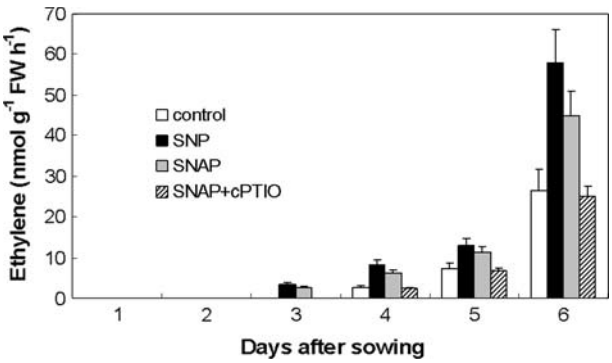

Fig. 3 Time course of ethylene emission of dormant apple embryos (control), embryos pretreated by SNP or SNAP (5 mM, 3 h) and embryos pretreated by SNAP (5 mM, 3 h) and imbibed in cPTIO. *Bars* are means ± SD of five replicates

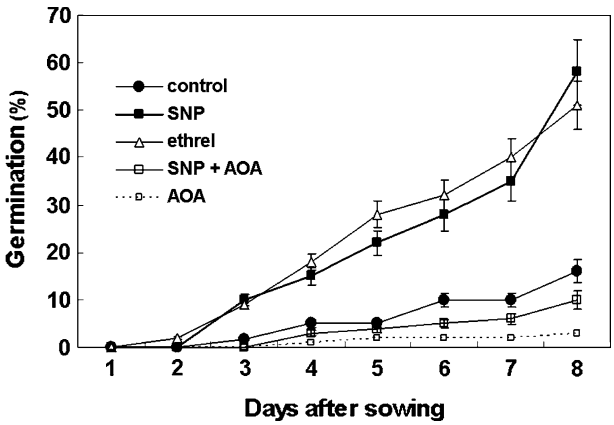

Fig. 4 Germination time courses of dormant apple embryos (control), control embryos imbibed in 1 mM ethrel (ethylene donor) or 1 mM AOA (inhibitor of ACC synthase), embryos pretreated with SNP (5 mM, 3 h) and embryos imbibed in 1 mM AOA after SNP pretreatment. Data point at each time are means ± SD of three replicates

the influence of ethrel vapors on dormancy breakage of apple embryos. Similar to imbibition with ethrel, its vapors removed embryo dormancy, and after 8 days 48% of embryos germinated (data not shown). Therefore, it was hypothesized that removing of embryonic dormancy in apple by NO-donors may be due to increased ethylene biosynthesis. SNP-pretreated embryos imbibed in 1 mM AOA, inhibitor of ACC synthase, did not germinate till 8th day. The AOA strengthened dormancy of untreated apple embryos (Fig. 4).

SNP decreases the sensitivity of apple embryos to exogenous ABA

ABA is required to maintain seed dormancy (Koornneef et al. 2002). It was tested if SNP modifies embryo sensitivity to exogenously added ABA. Germination of dormant (control) embryos was completely inhibited in the presence of 1–3 μM ABA. Lower concentration of ABA (0.1–0.3 μM) decreased germination to only 7 and 5%, respectively, after 8 days of imbibition (Fig. 5). However, imbibition in ABA of SNP pretreatment embryos only slightly decreased their germination (Fig. 5). More than 35 and 29% of embryos pretreated by SNP germinated after 8 days imbibition in 0.1 and 0.3 μM ABA, respectively (Fig. 5) and no morphological abnormalities of the seedlings were detected. The highest concentration ABA (3 μM) had to be used to total inhibition of germination of SNP pretreated embryos (Fig. 5).

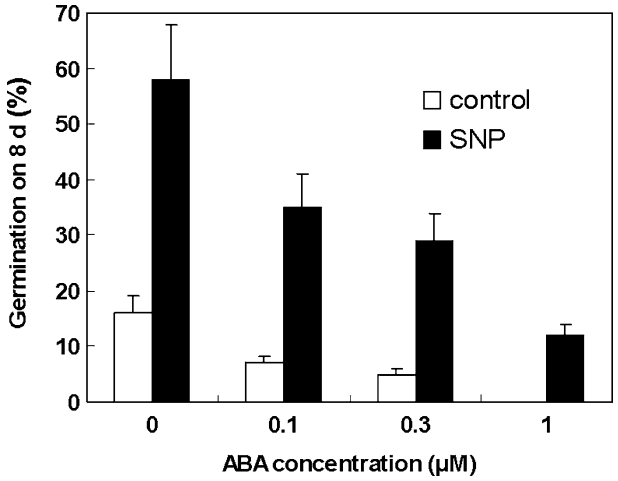

Fig. 5 Germination of dormant apple embryos (control) and embryos pretreated by SNP (5 mM, 3 h) imbibed in 0–1 μM ABA 8 days after sowing. *Bars* are means ± SD of three replicates

Discussion

Dormancy of apple seeds is expressed by inhibition of germination and several morphological abnormalities of young seedlings: inhibition of hypocotyls and internode elongation, asymmetric growth and greening of both cotyledons. All symptoms of embryonic dormancy of apple are removed as a result of 12-week-long cold stratification. Breakage of apple embryo dormancy is also observed in the presence of gibberellic acid (GA) (Smoleńska and Lewak 1971) or after HCN (1 mM, 6 h) pretreatment (Bogatek et al. 1991), but only the last treatment eliminates the asymmetry of cotyledons. Short pretreatment with NO donors affects germination of dormant apple embryos similarly to HCN, resulting in 3–4 stimulation of germination. cPTIO–NO scavenger reversed the stimulatory effect of NO-donor (Fig. 1) maintaining embryonic dormancy. The stimulatory effect of NO on seed germination and dormancy breakage was reported earlier by some authors. The majority of those data revealed light-dependent germination of seeds such as *Paulownia tomentosa* (Giba et al. 1998) or lettuce (Beligni and Lamattina 2000). It was suggested that NO acts as a signal molecule which is able to break phytochrome-regulated dormancy (Giba et al. 2003). Treatment with SNP also had a clear stimulating effect on germination of yellow lupine seeds (Kopyra and Gwóźdź 2003). Lupine seeds are neither photoblastic nor dormant seeds; therefore the role of NO is probably different as compared to lettuce. Simontacchi et al. (2004) reported the NO generation during early germination of Sorghum seeds. Since the increase in NO content preceded the initiation of phase II and the sharp oxygen consumption, the signal role of NO in seed germination was suggested. Our data supported such signaling role of NO in the regulation of germination process. Short-term (3 h) SNP or SNAP pretreatment is sufficient to break deep dormancy of apple embryo, resulting in undisturbed germination and normal seedling development, e.g., synchronic growth and greening of both cotyledons (Fig. 2). It is interesting that such an effect of NO-donors was not reported before. The results presented in this paper confirmed the morphogenic effect of NO donors, similar to that induced by HCN (Bogatek et al. 1991). Therefore we may suspect that NO acts as a signal molecule starting biochemical or molecular events leading to radicle growth and seedling development.

Ethylene is a plant hormone involved in seed germination (Kępczyński and Kępczyńska 1997 and references herein; Matilla 2000). Increasing ethylene evolution accompanies germination of most seeds (Petruzzelli et al. 2000 and references herein), but the role of ethylene in this process remains unclear. It is still questionable if ethylene emission is a consequence of germination, or in opposite, ethylene is necessary for undisturbed germination. In some species (peanut, sunflower) ethylene releases dormancy; in many other species, ethylene alone is not sufficient to release seed dormancy even when it promotes germination of non-dormant seeds of given species (Kucera et al. 2005 and references herein). The positive regulation of germination by ethylene evolved from ethrel solution was observed for dormant *Fagus silvatica* seeds (Calvo et al. 2004). Gaseous ethylene also removed dormancy and stimulated germination of *Stylosanthes humilis* seeds (Ribeiro and Barros 2004). As reported previously HCN pretreatment breaks dormancy of apple embryos (Bogatek et al. 1991, 2003). This stimulation of apple embryos germination after short time (6 h) HCN pretreatment was accompanied by increased emission of ethylene (Bogatek and Sykała 2005). Moreover, HCN induced the activity of ACC oxidase (ACO) and ACC synthase (ACS), two enzymes engaged in ethylene biosynthesis (Bogatek and Sykała 2005). Cyanide provoked a 2–3-fold increase in ACS activity and ACC level. Additionally, it was well correlated with a rapid increase in transcript levels of the ACS gene ACS6 (Bogatek and Sykała 2005). Parani et al. (2004) identified 342 unique genes upregulated in response to SNP treatment in *Arabidopsis*. For approximately, 96% of the genes a decline in average fold-change was observed with c-PTIO treatment, as compared to SNP. Authors also reported upregulation of

genes for ACC synthase and ACC oxidase in response to SNP pretreatment, but did not determine ethylene emission. One of the class of transcription factors induced by NO was the ethylene-responsive element-binding protein (EREBPs). SNP treatment induced transcripts coding for several EREBPs, by 2–13-fold over control expression (Parani et al. 2004). On the other hand, Leubner-Metzger et al. (1998) showed a novel pattern of EREBPs expression during germination of tobacco seeds. They demonstrated that expression of EREBP-3 was affected by ethylene and ABA and that transcriptional regulation of class I β-1, 3-glucanase (GLU I) induced prior to radicle emergence in tobacco depended partially on the activation of the ethylene-signaling pathways acting via EREBP-3. We noticed that exogenously applied ethylene removed dormancy of apple embryos (Fig. 4) resulting in enhanced germination of dormant embryos. Additionally, we observed an increase in emission of ethylene in embryos exposed for 3 h to 5 mM SNP or 5 mM SNAP (Fig. 3). Just after 3 days of culture ethylene production by those embryos was detected, while after 6 days ethylene production more than doubled as compared to the control (not germinating seeds). Experiments with AOA, the inhibitor of the ACC synthase, the key enzyme in the ethylene biosynthetic pathway, suggest that the NO donor influences germination by ethylene. Since ethylene counteracts ABA effects during seed germination (Kucera et al. 2005) we examined the effect of the NO donor on ABA inhibited germination of apple embryos. Our previous data demonstrated a continuous decrease in tissue ABA concentration during imbibition of dormant, control embryos in water (Bogatek et al. 2003). The decrease in ABA tissue level was not accompanied by an increase in embryo germinability, suggesting the involvement of other hormones/molecules in the regulation of removal of apple seed dormancy. Cyanide pretreatment of dormant embryos resulted in a sharp decrease in ABA concentration during the first 3 days of imbibition in water, and then a marked increase in hormone level was observed (Bogatek et al. 2003). This is in agreement with the hypothetical model of hormonal action on early seedling growth in *Arabidopsis* presented by Gazzarini and McCourt (2001). During germination "sensu stricto" seeds are highly sensitive to ABA, but when the seedlings become autotrophic the sensitivity to ABA decreases. Ethylene is a negative regulator of ABA in the seeds but may act as positive regulator of ABA during seedling growth (Beaudoin et al. 2000; Ghaseemian et al. 2000). ABA strengthens dormancy of control apple embryos, resulting in high inhibition of germination, while NO donor-treated embryos germinated well even in the presence of 0.3 μM exogenous ABA (Fig. 5). A similar result was reported by Bethke et al. (2006) for *Arabidopsis* seeds. SNP vapors decrease the sensitivity of *Arabidopsis* seeds to ABA, but norfluorazon, the inhibitor of ABA synthesis, was not sufficient to reduce the seed dormancy of *Arabidopsis*.

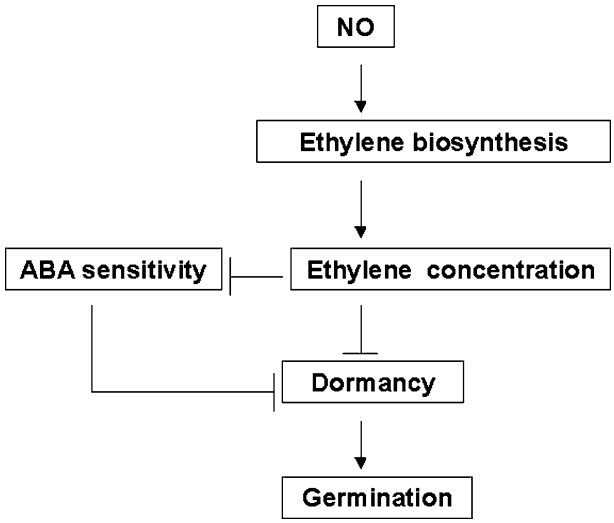

Fig. 6 Model illustrating the hypothetical function of NO in breaking the dormancy of apple embryos. NO increases ethylene biosynthesis and its concentration in seeds. Ethylene lowers seed sensitivity to ABA, thus promoting dormancy breakage and stimulating seed germination

NO-mediated loss of dormancy in apple embryos engages stimulation of ethylene biosynthesis. Since NO reduced the sensitivity of apple embryos to ABA it may be suggested that ethylene is a helpful factor possibly involved in the interaction between ABA and NO signaling pathways during induction of seed germination and seedling growth by NO donors (Fig. 6). Our results are in agreement with the previously postulated idea (Yamasaki 2005) that in plant physiology NO may act as a plant hormone equivalent to ethylene; that it is a gaseous signal transmitter.

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References

Beaudoin N, Serizet C, Gosti F, Giraudat J (2000) Interaction between abscisic acid and ethylene signaling cascades. Plant Cell 12:1103–1115

Beligni MV, Lamattina L (2000) Nitric oxide stimulates seed germination and de-etiolation, and inhibits hypocotyls elongation, three light-inducible responses in plants. Planta 210:215–221

Bethke PC, Gubler F, Jacobsen JV, Jones RL (2004) Dormancy of *Arabidopsis* seeds and barley grains can be broken by nitric oxide. Planta 219:847–855

Bethke PC, Libourel IGL, Jones RL (2006) Nitric oxide reduces seed dormancy in *Arabidopsis*. J Exp Bot 57:517–526

Biddle E, Kerfoot DGS, Kho YH, Russell KE (1976) Kinetic studies of the thermal decomposition of 2-chloroethyphosphonic acid in aqueous solution. Plant Physiol 58:700–702

Bogatek R (1995) The possible role of fructose 2,6-bisphosphate in the cyanide-mediated removal of embryonic dormancy in apple. Physiol Plant 94:460–464

Bogatek R, Sykała A (2005) Signaling action of cyanide on ethylene biosynthesis in dormant apple embryos. Eighth international workshop on seeds "germinating new ideas", Brisbane, Australia, pp 72

Bogatek R, Dziewanowska K, Lewak St (1991) Hydrogen cyanide and embryonal dormancy in apple seeds. Physiol Plant 83:417–421

Bogatek R, Come D, Corbineau F, Ranjan R, Lewak St (2002) Jasmonic acid affects dormancy and sugar catabolism in germinating apple embryos. Plant Physiol Biochem 40:167–173

Bogatek R, Gawrońska H, Oracz K (2003) Involvement of oxidative stress and ABA in CN-mediated elimination of embryonic dormancy in apple. In: Nicolas G, Bradford KJ, Come D, Pritchard HW (eds) The biology of seeds: recent research advances. CABI Publishing, Wallingford, pp 211–216

Calvo AP, Nicolas C, Lorenzo O, Nocolas G, Rodriguez D (2004) Evidence for positive regulation by gibberellins and ethylene of ACC oxidase expression and activity during transition from dormancy to germination in *Fagus sylvatica* L. seeds. J Plant Growth Regul 23:44–53

Correa-Aragunde N, Graziano M, Chevalier C, Lamattina L (2006) Nitric oxide modulates the expression of cell cycle regulatory genes during lateral root formation in tomato. J Exp Bot 57:581–588

Gazzarrini S, McCourt P (2001) Genetic interactions between ABA, ethylene and sugar signaling pathways. Curr Opin Plant Biol 4:387–391

Ghaseemian M, Nambara E, Cutler S, Kawaide H, Kamiya Y, McCourt P (2000) Regulation of abscisic acid signaling by the ethylene response pathway in *Arabidopsis*. Plant Cell 12:1117–1126

Giba Z, Grubisic D, Todorovic S, Sajc L, Stojakovic D, Konjevic R (1998) Effect of nitric oxide-releasing compounds on phytochrome-controlled germination of empress tree seeds. Plant Growth Regul 26:175–181

Giba Z, Grubisic D, Konjevic R (2003) Nitrogen oxides as environmental sensors for seed. Seed Sci Res 13:187–196

Hung KT, Kao CH (2003) Nitric oxide counteracts the senescence of rice leaves induced by abscisic acid. J Plant Physiol 160:871–879

Kępczyński J, Kępczyńska E (1997) Ethylene in seed dormancy and germination. Physiol Plant 101:720–726

Koornneef M, Bentisink L, Hilhorst H (2002) Seed dormancy and germination. Curr Opin Plant Biol 5:1–4

Kopyra M, Gwóźdź EA (2003) Nitric oxide stimulate seed germination and counteracts the inhibitory effect of heavy metals and salinity on root growth of *Lupinus luteus*. Plant Physiol Biochem 41:1011–1017

Kucera B, Cohn MA Leubner-Metzger G (2005) Plant hormone interactions during seed dormancy release and germination. Seed Sci Res 15:281–307

Lamattina L, Garcia-Mata C, Graziano M, Pagnussat G (2003) Nitric oxide: the versatility of an extensive signal molecule. Annu Rev Plant Biol 54:109–136

Leshem YY, Wills RBH, Veng-Va Ku V (1998) Evidence for the function of the radical gas-nitric oxide (NO) as an endogenous maturation and senescence regulating factor in higher plants. Plant Physiol Biochem 36:825–833

Leubner-Metzger G, Petruzzelli L, Waldvogel R, Vogeli-Lange R, Meins F Jr (1998) Ethylene-responsive element binding protein (EREBP) expression and the transcriptional regulation of class I β-1, 3-glucanase during tobacco seed germination. Plant Mol Biol 38:785–795

Matilla AJ (2000) Ethylene in seed formation and germination. Seed Sci Res 10:111–126

Murgia I, de Pinto MC, Delledonne M, Soave C, de Gara L (2004) Comparative effects of various nitric oxide donors on ferritin regulation, programmed cell death and cell redox state in plant cells. J Plant Physiol 161:777–783

Neill SJ, Desikan R, Clarke A, Hancock JT (2002) Nitric oxide is a novel component of abscisic acid signaling in stomatal guard cells. Plant Physiol 128:13–16

Pagnussat GC, Simontacchi M, Puntarulo S, Lamattina L (2002) Nitric oxide is required for root organogenesis. Plant Physiol 129:954–956

Parani M, Rudrabhatla S, Myers R, Weirich H, Smith B, Leaman DW, Goldman SL (2004) Microarray analysis of nitric oxide responsive transcripts in *Arabidopsis*. Plant Biotechnol J 2:359–366

Petruzzelli L, Coraggio I, Leubner-Metzger G (2000) Ethylene promotes ethylene biosynthesis during pea seed germination by positive feedback regulation of 1-aminocyclo-propane-1-carboxylic acid oxidase. Planta 211:144–149

Ribeiro DM, Barros RS (2004) Germination of dormant seed of *Stylosanthes humilis* as promoted by ethylene accumulation in closed environments. Braz J Plant Physiol 16:83–88

del Rio LA, Corpas FJ, Barroso JB (2004) Nitric oxide and nitric oxide synthase activity in plants. Phytochemistry 65:783–792

Scherer GFE (2000) NO donors mimic and NO inhibitors inhibit cytokinin action in betaine accumulation in *Amaranthus caudatus*. Plant Growth Regul 32:345–350

Seregelyes C, Barna B, Hennig J, Konopka D, Pasternak TP, Lukacs N, Feher A, Horvath GV, Dudits D (2003) Phytoglobins can interfere with nitric oxide functions during plant growth and pathogenic responses: a transgenic approach. Plant Sci 165:541–550

Simontacchi M, Jasid S, Puntarulo S (2004) Nitric oxide generation during early germination of sorghum seeds. Plant Sci 167:839–847

Smoleńska G, Lewak St (1971) Gibberellins and the photosensitivity of isolated embryos from non-stratified apple seeds. Planta 99:144–153

Yamasaki H (2005) The NO world for plants: achieving balance in an open system. Plant Cell Environ 28:78–84