INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
16–344/2017/19–1–41–47
DOI: 10.17957/IJAB/15.0236
http://www.fspublishers.org

Full Length Article

High-throughput Screening of Rice for Nitrosative-stress Response and the Identification of Effective pH Range for Nitric Oxide Donor S-Nitrocysteine

Bong-Gyu Mun[1], Chan-Ju Lee[1], Adil Hussain[1,2], Gang Sub Lee[3], Sang-Uk Lee[1], Kyung-Min Kim[1] and Byung-Wook Yun[1*]

[1]*School of Applied Biosciences, Kyungpook National University, Daegu, Republic of Korea*
[2]*Department of Agriculture, Abdul Wali Khan University, Mardan, Pakistan*
[3]*Biosafety Division, National Institute of Agricultural Sciences, Jeonju 54874, Korea*
[*]For corresponding author: bwyun@knu.ac.kr

Abstract

Nitric oxide (NO)-mediated signaling regulates growth, development, and responses to biotic and abiotic stresses in plants. It involves various NO-derivatives, such as NO radical, nitroxyl anion (NO^-), peroxynitrite ($ONOO^-$), and *S*-nitrosothiols (SNOs). Many of these SNOs including S-nitrosocysteine (CysNO), can modify intracellular protein thiols through trans-nitrosation reactions. Various NO donors have been used as tools to unravel the fundamental mechanisms of NO signaling. However, the effect of NO donors can be analyzed only under specific conditions. Here, we report a high-throughput screening system for identifying mutant lines exhibiting differential responses to the frequently used NO donor CysNO. To determine the effect of CysNO on seed germination and growth at various pH levels, we grew *Arabidopsis thaliana* seeds on MS medium with various concentrations of CysNO at a pH range of 2–5.8. The pH was adjusted using different volumes of HCl. Seeds were found to be highly sensitive to pH below 4.5 at any given CysNO concentration; therefore, the effects of the CysNO treatments could not be determined. A similar effect of pH was observed in the case of rice plants. However, when the same concentrations of CysNO were prepared using EPPS (3-[4-(2-Hydroxyethyl)-1-piperazinyl] propanesulfonic acid) buffer to maintain pH at a suitable level, significantly different responses to CysNO were observed among wild-type and mutant rice lines, indicating the importance of optimum pH conditions. Using this technique, we identified several rice *Ac/Ds* transposon mutant lines that showed tolerance or sensitivity to exogenous NO stress. In conclusion, pH is a critical factor for plant germination and growth. However, information about changes of pH caused CysNO solution was lacking. In this study we demonstrated the effective pH range for maximum efficiency and absorbance of CysNO using EPPS buffer system in rice. Hence, the EPPS-based buffer system is more efficient for high-throughput screening in rice against nitrosative stress induced by the nitric oxide donor S-Nitrocysteine. © 2017 Friends Science Publishers

Keywords: Nitric oxide; CysNO; EPPS buffer; pH; High-throughput screening

Introduction

Nitric oxide (NO), a gaseous, redox-active small molecule, modulates a plethora of physiological functions in plants. This small gaseous redox active molecule has gained much importance because of its tremendous role in plant growth development, and signaling. Most of the pioneering research on NO in plants focused on analyzing its toxic effects on the photosynthetic apparatus and other physiological functions. Since the discovery of NO as a signaling molecule in plants during the late 1990s, intensive research has been conducted to identify its roles in various biomolecular pathways (Arasimowicz and Wieczorek, 2007). Studies have revealed that NO is involved in stimulation of seed germination (Beligni and Lamattina, 1999), plant maturation and senescence (Ya'acov *et al.*, 1998; Guo and Crawford, 2005), stomatal regulation (Neill *et al.*, 2002; Bright *et al.*, 2006), abiotic stress tolerance (Corpas *et al.*, 2011; Khan *et al.*, 2012) and plant immunity (Yoshioka *et al.*, 2011). Its involvement in so many biological processes, its simple diatomic structure and highly reactive nature enables it to form various complexes with other cellular components (Bogdan, 2001). NO-mediated signaling is potentiated by various NO derivatives, such as NO radical, nitroxyl anion(NO^-), peroxynitrite ($ONOO^-$), and *S*-nitrosothiols (SNOs). Many redox signaling pathways mediated by these NO derivatives rely on target specificity and proper substrate processing to ensure appropriate signaling. For example, NO binds to solvent-exposed cysteine (*Cys*) thiols of various proteins through a redox-sensitive covalent bond to form SNOs, a phenomenon known as S-nitrosylation (Nathan, 2003). This post-translational modification (PTM)

To cite this paper: Mun, B.G., C.J. Lee, A. Hussain G.S. Lee, S.U. Lee, K.M. Kim and B.W. Yun, 2017. High-throughput screening of rice for nitrosative-stress response and the identification of effective pH range for nitric oxide donor S-nitrocysteine. *Int. J. Agric. Biol.*, 19: 41–47

affects the structure of the target protein. *S*-nitrosylation is a key mechanism for regulating protein function at the cellular level (Wiseman and Halliwell, 1996). Many other types of PTMs (such as phosphorylation, glycosylation, ubiquitination, methylation, acetylation, and lipidation) play important roles in the regulation of gene function (Cain *et al*., 2014), protein degradation (Geiss-Friedlander and Melchior, 2007), cellular differentiation (Grotenbreg and Ploegh, 2007), and signaling (Morrison *et al*., 2002; Jensen, 2004). S-nitrosylation is considered one of the most important PTMs because of the high reactivity of protein thiols and the influence of *S*-nitrosylation on protein functions under various basal and induced physiological conditions (Vanin *et al*., 1997; Nathan, 2003; Martinez-Ruiz and Lamas, 2004). The role of SNOs in plant disease resistance has been established (Feechan *et al*., 2005). Cellular levels of SNOs are controlled by the enzyme S-nitrosoglutathione reductase (*GSNOR*) through the process of de-nitrosylation (Malik *et al*., 2011).

Several types of NO donors have been used in experiments to ascertain the roles of NO and other reactive nitrogen species in modulating different cellular processes. The application of exogenous NO to plants has unearthed the diverse roles of NO in cell wall lignification, cell death, regulation of guard cells, and senescence (Takahashi and Yamasaki, 2002; Hung and Kao, 2003). Synthetic *S*-nitroso-L-cysteine (CysNO) is a reactive, highly diffusible low-molecular-weight NO source that has been used as an NO donor in many different studies (Gu and Lewis, 2007; Terrile *et al*., 2013). Translocation of CysNO into cells, leads to a global increase in SNO levels and the initiation of S-nitrosylation. Thus, CysNO can be used to induce nitrosative stress *in vitro*. It can also be used to manipulate intracellular protein thiols through trans-nitrosation; therefore, it is an important tool for unraveling the fundamental mechanisms of NO signaling. CysNO can be applied to MS medium or other synthetic media for seed testing or can be infiltrated into the leaf apoplast. In a variety of scientific studies published and available in the literature, CysNO has been prepared by mixing equimolar amounts of L-*Cysteine* and sodium nitrate in HCl (Zhang and Hogg, 2004; Lam *et al*., 2010). However, this results in a highly acidic solution, which may result in reducing the efficiency of NO absorption or even lead to complete decomposition (D'Ulivo *et al*., 2004; Gu and Lewis, 2007). Additionally, the highly acidic nature of the resulting CysNO solution may affect the substrate and/or other experimental materials and confound the results. Low or high pH of synthetic media also influences seed germination and negatively affects early plant development (Jansen and Cronin, 1953; Shoemaker and Carlson, 1990; Chohura *et al*., 2004). In addition, absorption of macro- and micro-nutrients from the substrate is determined by pH (Salter and McIlvaine, 1920; McCall, 1980; Kabata-Pendias and Pendias, 1999). Therefore, pH is an important factor to consider when working with CysNO. The efficiency of various NO donors such as S-nitrosoglutathione (GSNO), sodium nitroprusside (SNP) and CysNO in various screening experiments and their effect on seed and/or plant physiology has not been investigated so far at different pH regimes. Using two different model plant systems i.e. *Arabidopsis thaliana* and rice, this study presents an EPPS buffer-based system for high throughput screening of plants under nitrosative stress induced by the NO donor CysNO. Consequently, CysNO mediated low pH resulted in inhibition of plant germination and growth thus proper range of pH on CysNO application is essential and proper buffering is recommended. This is hypothesized that this buffer-system may prove helpful and more efficient for such studies in future.

Materials and Methods

Plant Material

Wild-type *Arabidopsis thaliana* seeds (ecotype Col-0) were used to assess damage caused by low pH and different concentrations of CysNO solution. Seeds were sterilized with a solution of 50% bleach (including 0.1% Triton X-100) and chilled at 4°C for 24 h before plating on 1/2 MS medium. For rice experiments, 124 *Ac*/*Ds* transposon mutant lines (Dongjin background) were obtained from the Rural Development Administration (RDA), Jeonju-si, Korea, and all mutant lines were screened for their response to nitrosative stress. Rice seeds were surface sterilized in 25% prochloraz and germinated on sterile tissue paper in the dark for 4 days. Seedlings were then incubated for 10 days under normal growth conditions (16 h light/8 h dark at 23°C).

Evaluating the Effect of pH with MS Medium

To evaluate pH effect on seed germination, different concentration of MS medium were made by adding different volumes (75, 200 and 375 µL) of 50 mM CysNO solution to 25 mL of autoclaved MS and pH was checked with pH indicator paper (Advantec, UNIV) before the medium solidified. Additionally, to generate 0.75 mM mM of CysNO contained MS medium, different concentration of CysNO stock solutions (125 mM, 250 mM, and 500 mM) were prepared by mixing equal amounts of L-cysteine solution and $NaNO_2$ solution. Twelve to 15 surface-sterilized Arabidopsis seeds were sown on each MS plate. The approximate pH of all media was recorded referring from pH indicator strips. Any effects of CysNO and substrate-pH on the plants were monitored and recorded regularly. All experiments were setup in triplicates and included appropriate control treatments.

High-throughput Screening for Nitrosative Stress

Rice Ac/Ds mutant lines were evaluated for their response to nitrosative stress. For this purpose, rice seeds were grown in

dark for 4 days. Seedlings were then incubated for 10 days under normal growth conditions (16 h light/8 h dark at 23°C). The seedlings were then incubated in 6-well plates containing CysNO solution at different final concentrations (10 mM, 25 mM and 50 mM). Plates were kept in dark for 72 h and then transferred to normal conditions (16 h light and 8 h dark). Parallel experiments were set up using CysNO solutions of the same concentrations made in EPPS buffer (40 mM EPPS pH 7.6) to determine the effect of pH on the absorption of CysNO by the plants and the subsequent response of plants to nitrosative stress. All plates were setup in triplicates along with appropriate control plates.

RNA Extraction and RT-PCR

RT-PCR was performed to determine the expression of NO-responsive genes in the wild-type rice cultivar Dongjin. Total RNA was extracted from rice leaf tissues using an RNeasy Plant Mini Kit (Qiagen). Complementary DNA was synthesized using an Omniscript-RT Kit (Qiagen). All the reactions were performed according to the manufacturer's instructions. RT-PCR was conducted for 3 nitrosative stress response genes (nitric oxide associated 1 (*OsNOA1*), ascorbate peroxidase 1 (*OsAPX1*), and catalase A (*OsCATA*)) and the actin gene (*OsActin*), using the primers listed in Table 3.

Results

Effect of Low HCl-mediated pH on Seed Germination

Several experiments with CysNO have shown that the impact of this NO donor is highly dependent upon avoidance of its decomposition from light and pH for its uses (Ravinder *et al.*, 1996; Gu and Lewis, 2007). CysNO solution made with HCl led to inconsistencies in many of the experiments conducted in our laboratory and elsewhere. Therefore, in this study, to examine the effect of pH on the efficacy of NO donor absorption and on the plants. The CysNO solution with usual method using HCl (Zhang and Hogg, 2004). The pH of the MS medium was determined with pH indicator paper (Fig. 1). Fig. 1 shows changes of pH value in MS media containing different final concentrations of CysNO and HCl (Table 1). Germination of Arabidopsis seeds (Col-0) was completely prohibited on MS at pH 4 and below. On plates with a pH value of 4, the final concentrations of CysNO and HCl were 0.4 mM and 4 mM, respectively (Fig. 1). On plates with 0.75 mM CysNO, the HCl concentration was 7.5 mM, resulting in a pH of 2, which completely prevented seed germination (Fig. 1). In contrast, 100% seed germination was observed at pH 5 and 5.8 (Fig. 1A & B). Next, an experiment was conducted to determine whether the germination inhibition was due to low HCl mediated pH or CysNo. Seeds were grown on plates at the highest concentration of CysNO (0.75 mM). This concentration was fixed for all plates by treating with different concentration CysNO stock solutions (500 mM, 250 mM and 125 mM) (Table 2). This high concentration of stock solution only small volumes was required to achieve a final concentration of 0.75 mM CysNO in the MS medium, thus reducing the amount of HCl in the medium. Therefore, a high rate of seed germination was achieved on plates with 0.75 mM CysNO and (Fig. 2-B), since the pH (4.5) was much higher than that in the previous experiment at the same concentration of CysNO. Germination of Arabidopsis seedling was completely prevented at higher concentrations of HCl (Fig. 2C & D).

Development of a High-throughput Method for Evaluating Plant Responses to CysNO

HCl-mediated low pH had adverse effects on germination of Arabidopsis seeds and because of low pH, effect of CysNO could not be observed accurately. This necessitated the development of a new method to eliminate the negative effects of low pH and study the effects of CysNO on wild type and rice mutant plants. For this purpose, wild-type and mutant seeds of rice were germinated in the dark for 4 days and then grown under normal growth conditions (16 h light/8 h dark at 23°C). After 2 weeks of growth, plants were transferred to 6-well plates containing CysNO solution at different concentrations (10 mM, 25 mM and 50 mM). Control plants were transferred to plates containing only distilled water. Plates were kept in the dark for 72 h and then transferred to normal conditions. Interestingly, all rice seedlings treated with HCl-CysNO turned yellow and chlorotic. Symptoms on plant started to appear within 12 h after the treatment and became severe after 24 h. Evidently, this was caused by the extremely low pH of 2 (Fig. 3A & B). These results are consistent with previous findings that low pH negatively affects plant development (Petra and John, 2000). Damage induced by low pH was minimized when the HCl concentrations were reduced, thereby increasing the pH (Fig. 3C & D). These results are consistent with our previous findings using Arabidopsis seeds, where germination was inhibited due to low HCl-mediated pH. To minimize the negative effects of low HCl-mediated substrate pH on the plants, we set up parallel experiments using CysNO solutions of the same concentrations made with 40 mM EPPS buffer. Damage induced by low pH was minimized when the HCl concentrations were reduced with EPPS buffering, thereby increasing the pH (Fig. 3E & F). Significant changes in the pH of the medium were observed, and a pH of 5 was recorded at the highest concentration of CysNO (50 mM), at which all rice plants had good growth. Using the same method, we observed significant differences in growth and other characteristics among wild-type and mutant rice lines, indicating that this method significantly increased the reliability and robustness of the experiments.

Table 1: Volumes of 50 mM CysNO stock solution added to Murashige and Skoog (MS) medium and the effect on substrate pH and seed germination

Concentration of CysNO stock solution (mM)	Volume of CysNO solution added to 25 mL MS (µL)	Final concentration of CysNO in 25 mL MS (mM)	Final concentration of HCl in 25 mL MS (mM)	pH of the medium	Seed germination
0	0	0	0	5.8	Yes
50	75	0.15	1.5	5	Yes
50	200	0.4	4	4	No
50	375	0.75	7.5	2	No

Table 2: Volumes of high-concentration CysNO stock solutions added to Murashige and Skoog (MS) medium to obtain a final CysNO concentration of 0.75 mM

Concentration of CysNO stock solution (mM)	Volume of CysNO solution added to 25 mL MS (µL)	Final concentration of CysNO in 25 mL MS (mM)	Final concentration of HCl in 25 mL MS (mM)	pH of the medium	Seed germination
0	0	0	0	5.8	Yes
500	37.5	0.75	0.5	4.5	Yes
250	75	0.75	1.5	4	Yes
125	150	0.75	3	2	No

Table 3: List of genes analyzed by RT-PCR

No.	Gene	Primers sequences	
1	*OsNOA1* (LOC_Os02g01440)	Forward: AGAGAAGTTGGAGTTACATTGAC;	Reverse: CATACTATGCATAGAAAT-GGAGAC
2	*OsAPX1* (LOC_Os03g17690)	Forward: CCAAGGGTTCTGACCACCTA;	Reverse: CAAGGTCCCTCAAAACCAGA
3	*OsCATA* (LOC_Os02g02400)	Forward: CGGATAGACAGGAGAGGTTCA;	Reverse: AATCTTCACCCCCAACGACT
4	*OsActin*	Forward: GGAACTGGTATGGTCAAGGC;	Reverse: AGTCTCATGGATACCCGCAG

Effect of EPPS Buffer on the Efficiency of Exogenous NO Absorption

The stringency of the high-throughput screening was assessed in real time by analyzing the expression of oxidative and nitrosative stress response genes in wild-type rice plants. In general, Low pH accumulate H^+ in plant and it triggers oxidative stress, through the production of superoxide radicals (O_2^-•) and hydrogen peroxide (H_2O_2) in plant tissues. For this reason, expression of rice *OsNOA1*, *OsAPX1* and *OsCATA* was analyzed after 12, 24, 48 and 72 h of treatment with CysNO + 40 mM EPPS buffer. Clear variations in gene expression were observed among CysNO concentrations for all of these genes, indicating that nitrosative stress was achieved using CysNO solution prepared with EPPS buffer. The expression of *OsNOA1* and *OsCATA* increased in response to treatment with 10 mM and 25 mM EPPS-CysNO treatment, while expression of *OsAPX1* increased only after treatment with 25 mM EPPS-CysNO treatment (Fig. 4). In contrast, when expression of the same set of genes was analyzed in plants treated with the same concentration of CysNO solutions (but without EPPS), a high degree of similarity was observed in expression pattern of *OsAPX1* among all concentrations of HCl-CysNO, rendering CysNO application as non-significant (Fig. 4A).

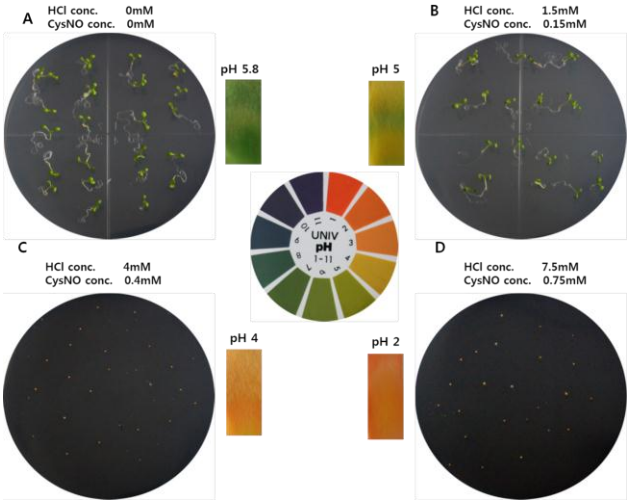

Fig. 1: Low pH mediated by HCl in CysNO solution affects Arabidopsis seed germination

Arabidopsis thaliana seeds successfully germinated on Murashige and Skoog (MS) plates with HCl concentrations of 0 and 1.5 mM (pH 5.8 and 5, respectively) (A, B). However, on medium supplemented with 4 or 7.5 mM HCl, Arabidopsis seeds failed to germinate, as the pH of these plates was 4 and 2, respectively (C, D). As a result of this low pH, the effects of CysNO treatment on the seeds at low pH were insignificant

Discussion

Different NO sources, such as SNP (sodium nitroprusside), GSNO (S-nitrosoglutathione) and CysNO are used to investigate the role of NO in plant signaling pathways. Among these NO source, CysNO is the simplest and most reactive NO donor and a critical experimental tool for studying the fundamental mechanisms of NO signaling in plants (Gu and Lewis, 2007). However, CysNO must be handled carefully because its decomposition rate can be

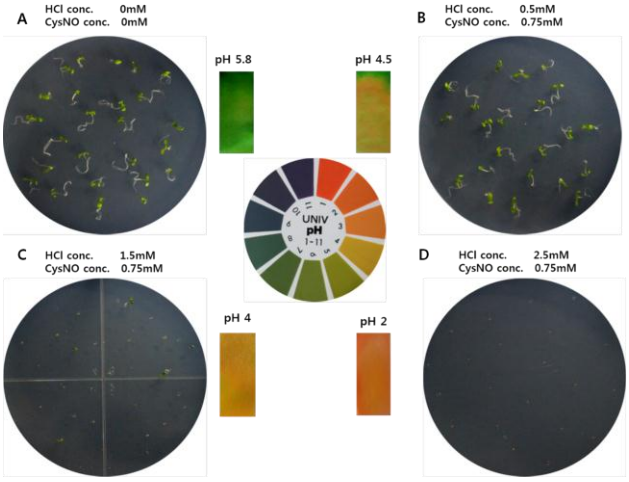

Fig. 2: Reducing the concentration of HCl helps to minimize the damage associated with low pH on seeds germination

Reducing the final concentration of HCl by using high-concentration CysNO stock solutions helps avoid damage to seeds caused by low pH. In contrast to the experiment shown in Fig. 1, in this experiment 100% germination was achieved at 0.75 mM CysNO (pH 4.5) (B). At pH 4 few seeds were germinated (C) and lower than pH 4 all seeds failed to germinate (D)

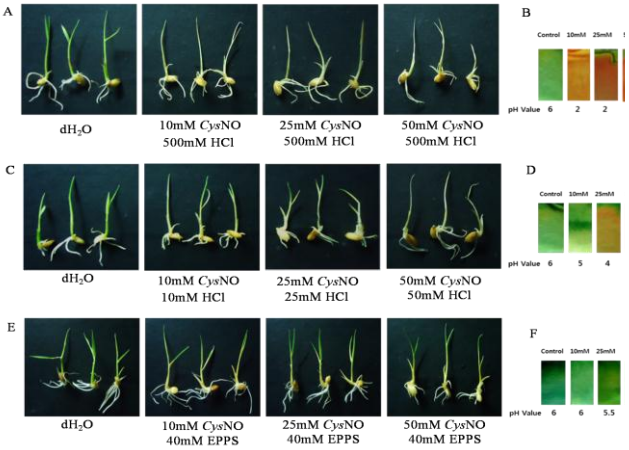

Fig. 3: CysNO solution with EPPS buffering minimize pH changes and reduce damage associated with low pH

Keeping the final concentration of HCl fixed (500 mM) at all concentrations of CysNO (10 mM, 25 mM, and 50 mM) resulted in no differences among treatments owing to extremely low pH (A, B). Likewise, no differences among CysNO treatments were observed when equimolar concentrations of CysNO and HCl were used (C, D). However, by using EPPS buffer, it was possible to achieve CysNO concentrations as high as 50 mM while keeping the pH at a suitable level of 5 to 6 (E, F)

affected by light and pH. CysNO is produced by mixing solution of L-cysteine and sodium nitrate. Hydrochloric acid is needed to dissolving L-cysteine. However, it affects the pH of the CysNO solution and any the substrate to which the solution is added. Previous research on CysNO revealed that this NO donor decomposes quickly at a pH of 6–8, whereas it is stable in acidic (pH < 5) and alkaline (pH > 9) conditions (Gu and Lewis, 2007). Therefore, changes of in pH must be considered when CysNO is being used. Additionally, extremely low or high pH values may affect seed germination and plant development. Recent researches showed that the growth of Arabidopsis roots was inhibited significantly at acidic or basic pH, and young seedlings turned yellow-brown or brown and subsequently died (Kang *et al.*, 2013). Additionally, low pH induces H^+ activity and it results in the production of superoxide and hydrogen peroxide in plant tissues. Recently published research indicated that increased production of superoxide results in decomposition of S-nitrosothiols in time dependent manner (Samir *et al.*, 1998; Hogg, 2002). Similar results were observed in our experiments; seed germination was significantly inhibited at around pH 4 or below pH 4 confirming that pH affects plant seed germination and development. This result suggested that low pH mediated by HCl affected plant seed germination and development. So far there have been a few studies clarifying the correlation of pH with the efficacy of CysNO application. This study revealed that the amount or concentration of CysNO can affect the substrate pH changes and plant responses to CysNO are highly dependent upon substrate pH. Additionally, low pH may negatively effect on plant growth and germination. Based on the experiments with Arabidopsis and rice substrate pH 2 or below, results in chlorosis. In previous studies, the amount of HCl in the substrate (and hence the pH of the substrate) could only be controlled by limiting the concentrations of CysNO. However, not many information was available on the optimization of media or buffers for the direct application of NO donors to plants or seeds. In the present study, the effects of traditionally made HCl-CysNO and EPPS-CysNO applications on Arabidopsis and rice seeds were compared. Our results showed that application of EPPS-CysNO permitted visual assessment of the effect of NO within a short time. Nevertheless, to verify these results, we determined the expression levels of genes known to be involved in plant responses to acid stress (*OsAPX1*, *OsCATA*) and NO stress (*OsNOA1*). According to previous research, gene expression of APX1 was up-regulated to contribute to the adaption in low pH condition. Meanwhile, CATA and CATB gene expression were significantly down-regulated after low pH exposure (Zhang *et al.*, 2015). In our results, APX1 and CATA gene expression were showed parallel patterns. This can support that HCl, the essential material for making CysNO solution affect pH of it thus, maintaining of pH for the solution must be considered.

Conclusion

Synthesis of CysNO solution in HCl not only reduces its efficiency and absorption capacity in plants but also induces

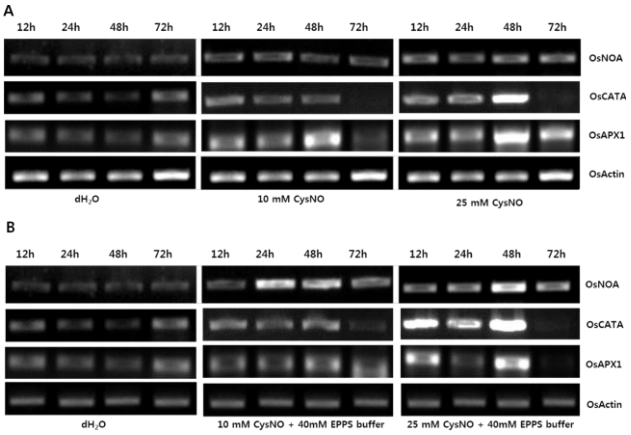

Fig. 4: Expression of nitrosative and oxidative stress response genes in plants treated with CysNO and EPPS-CysNO

(A) Gene expression in plants treated with CysNO was highly similar among the different time points of 12, 24, 48, and 72 hr. The expression of *OsAPX1* and *OsCATA* was higher in plants treated with 25 mM CysNO than in plants treated with 10 mM CysNO or control plants. (B) More robust and clear gene expression patterns were observed when plants were treated with EPPS-CysNO. Increases in the expression of *OsNOA1*, *OsCATA*, and *OsAPX1* were observed in plants treated with either 10 mM or 25 mM EPPS-CysNO

negative effects on the physiology of plants and/or seeds. Furthermore, efficiency of exogenous NO absorption with EPPS buffer was verified through nitrosative responsive genes (*OsNOA1, OsAPX1 and OsCATA*) expression. Taken together EPPS buffering helps to reduce low pH damage and increase CysNO absorption capacity plant. Therefore, we recommend the formulation of CysNO donor using the above mentioned EPPS buffer for high-throughput screening of rice under nitrosative stress conditions.

Acknowledgments

This work was supported by a grant from the Next-Generation BioGreen 21 Program (SSAC, Grant No. PJ01110201), Rural Development Administration, Republic of Korea.

References

Arasimowicz, M. and J.F. Wieczorek, 2007. Nitric oxide as a bioactive signalling molecule in plant stress responses. *Plant Sci.*, 172: 876–887

Beligni, M.V. and L. Lamattina, 1999. Nitric oxide counteracts cytotoxic processes mediated by reactive oxygen species in plant tissues. *Planta*, 208: 337–344

Bogdan, C., 2001. Nitric oxide and the regulation of gene expression. *Trends Cell Biol.*, 11: 66–75

Bright, J., R. Desikan, J.T. Hancock, I.S. Weir and S.J. Neill, 2006. ABA-induced NO generation and stomatal closure in Arabidopsis are dependent on H_2O_2 synthesis. *Plant J.*, 45: 113–122

Cain, J.A., N. Solis and S.J. Cordwell, 2014. Beyond gene expression: the impact of protein post-translational modifications in bacteria. *J. Proteomics*, 97: 265–286

Chohura, P., A. Komosa and E. Kołota, 2004. Wpływ pH pożywek na dynamikę zawartości makroelementów w liściach pomidora szklarniowego uprawianego na wełnie mineralnej. *Rocz. AR Pozn.*, 37: 29–35

Corpas, F.J., M. Leterrier, R. Valderrama, M. Airaki, M. Chaki, J.M. Palma and J.B. Barroso, 2011. Nitric oxide imbalance provokes a nitrosative response in plants under abiotic stress. *Plant Sci.*, 181: 604–611

D'Ulivo, A., M. Onor and E. Pitzalis, 2004. Role of hydroboron intermediates in the mechanism of chemical vapor generation in strongly acidic media. *Anal. Chem.*, 76: 6342–6352

Feechan, A., E. Kwon, B.W. Yun, Y. Wang, J.A. Pallas and G.J. Loake, 2005. A central role for *S*-nitrosothiols in plant disease resistance. *Proc. Natl. Acad. Sci. USA*, 102: 8054–8059

Geiss-Friedlander, R. and F. Melchior, 2007. Concepts in sumoylation: a decade on. *Nat. Rev. Mol. Cell Biol.*, 8: 947–956

Grotenbreg, G. and H. Ploegh, 2007. Chemical biology: dressed-up proteins. *Nature*, 446: 993–995

Gu, J. and R.S. Lewis, 2007. Effect of pH and metal ions on the decomposition rate of *S*-nitrosocysteine. *Ann. Biomed. Eng.*, 35: 1554–1560

Guo, F.Q. and N.M. Crawford, 2005. Arabidopsis nitric oxide synthase1 is targeted to mitochondria and protects against oxidative damage and dark-induced senescence. *Plant Cell*, 17: 3436–3450

Hung, K.T. and C.H. Kao, 2003. Nitric oxide counteracts the senescence of rice leaves induced by abscisic acid. *J. Plant Physiol.*, 160: 871–879

Hogg, N., 2002. The biochemistry and physiology of S-nitrosothiols. *Annu. Rev. Pharmacol. Toxicol.*, 42: 585–600

Jansen, L. and E. Cronin, 1953. Halogeton on trial. *Farm Home Sci.*, 14: 38–39

Jensen, O.N., 2004. Modification-specific proteomics: characterization of post-translational modifications by mass spectrometry. *Curr. Opin. Chem. Biol.*, 8: 33–41

Kabata-Pendias, A. and H. Pendias, 1999. *Biogeochemia Pierwiastków Śladowych*. PWN, Warsaw, Poland

Kang, T.T., N.A. Kari, B.H. Maxwell and J.D. Soquila, 2013. The effect of various pH levels on the root growth of *Arabidopsis thaliana* seedlings. *Expedition*, 2: 1–15

Khan, M.N., M.H. Siddiqui, F. Mohammad and M. Naeem, 2012. Interactive role of nitric oxide and calcium chloride in enhancing tolerance to salt stress. *Nitric Oxide*, 27: 210–218

Lam, Y.W., Y. Yuan, J. Isaac, C.V.S. Babu, J. Meller and S.M. Ho, 2010. Comprehensive identification and modified-site mapping of S-nitrosylated targets in prostate epithelial cells. *PLoS One*, 5: e9075

Malik, S.I., A. Hussain, B.W. Yun, S.H. Spoel and G.J. Loake, 2011. *GSNOR*-mediated de-nitrosylation in the plant defence response. *Plant Sci.*, 181: 540–544

Martinez-Ruiz, A. and S. Lamas, 2004. *S*-nitrosylation: a potential new paradigm in signal transduction. *Cardiovasc. Res.*, 62: 43–52

McCall, W.W., 1980. *The pH Preference of Plants*. General Home Garden Series 18, University of Hawaii, Honolulu, Hawaii, USA

Morrison, R.S., Y. Kinoshita, M.D. Johnson, T. Uo, J.T. Ho, J.K. McBee, T.P. Conrads and T.D. Veenstra, 2002. Proteomic analysis in the neurosciences. *Mol. Cell Proteomics*, 1: 553–560

Nathan, C., 2003. Specificity of a third kind: reactive oxygen and nitrogen intermediates in cell signaling. *J. Clin. Invest.*, 111: 769–778

Neill, S.J., R. Desikan, A. Clarke and J.T. Hancock, 2002. Nitric oxide is a novel component of abscisic acid signaling in stomatal guard cells. *Plant Physiol.*, 128: 13–16

Petra, S.K. and P. John, 2000. Why plants grow poorly on very acid soil: are ecologists missing the obvious? *J. Exp. Biol.*, 52: 791–799

Ravinder, J.S., N. Hogg, J. Joseph and B. kalyanaraman, 1996. Mechanism of Nitric Oxide Release from S-Nitrosothiols. *J. Biol Chem.*, 31: 18596–18603

Salter, R.M. and T. McIlvaine, 1920. Effect of reaction of solution on germination of seeds and on growth of seedlings. *J. Agric. Res.*, 19: 73–95

Samir, A., E. Milo., Y. Rose and P. Kostka, 1998. Superoxide-mediated decomposition of biological S-Nitrosothiols. *J. Biol Chem.*, 11: 6041–6045

Shoemaker, C.A. and W.H. Carlson, 1990. pH affects seed germination of eight bedding plant species. *HortScience*, 25: 762–764

Terrile, M.C., R. Paris, L.I.A.C. Villalobos, M.J. Iglesias, L. Lamattina, M. Estelle and C.A. Casalongue, 2013. Nitric oxide influences auxin signaling through S-nitrosylation of the Arabiposis Transport inhibitor response1 auxin receptor. *Plant J*., 3: 492–500

Takahashi, S. and H. Yamasaki, 2002. Reversible inhibition of photophosphorylation in chloroplasts by nitric oxide. *FEBS Lett*., 512: 145–148

Vanin, A.F., I.V. Malenkova and V.A. Serezhenkov, 1997. Iron catalyzes both decomposition and synthesis of *S*-nitrosothiols: optical and electron paramagnetic resonance studies. *Nitric Oxide*, 1: 191–203

Wiseman, H. and B. Halliwell, 1996. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochem. J*., 313: 17–29

Ya'acov, Y.L., R.B. Wills and V.V.V. Ku, 1998. Evidence for the function of the free radical gas—nitric oxide (NO•)—as an endogenous maturation and senescence regulating factor in higher plants. *Plant Physiol. Biochem*., 36: 825–833

Yoshioka, H., K. Mase, M. Yoshioka, M. Kobayashi and S. Asai, 2011. Regulatory mechanisms of nitric oxide and reactive oxygen species generation and their role in plant immunity. *Nitric Oxide*, 25: 216–221

Zhang, Y.K., D.F. Zhu, Y.P. Zhang, H.Z. Chen, J. Xiang and X.Q. Lin, 2015. Low pH-induced changes of antioxidant enzyme and ATPase activities in the roots of rice (*Oryza sativa* L.) Seedlings. *PLoS One*, 10: e0116971

Zhang, Y.H. and N. Hogg, 2004. The mechanism of transmembrane S-nitrosothiol transport. *PNAS*, 21: 7891–789

(Received 12 July 2016; Accepted 20 October 2016)