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RAPID COMMUNICATION

Glutathione synthesis is regulated by nitric oxide in *Medicago truncatula* roots

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Abstract Glutathione (GSH) is one of the main antioxidants in plants. Legumes have the specificity to produce a GSH homolog, homoglutathione (hGSH). We have investigated the regulation of GSH and hGSH synthesis by nitric oxide (NO) in *Medicago truncatula* roots. Analysis of the expression level of gamma-glutamylcysteine synthetase (γ-ECS), glutathione synthetase (GSHS) and homoglutathione synthetase (hGSHS) after treatment with sodium nitroprusside (SNP) and nitrosoglutathione (GSNO), two NO-donors, showed that *γ-ecs* and *gshs* genes are up regulated by NO treatment whereas *hgshs* expression is not. Differential accumulation of GSH was correlated to gene expression in SNP-treated roots. Our results provide the first evidence that GSH synthesis pathway is regulated by NO in plants and that there is a differential regulation between *gshs* and *hgshs* in *M. truncatula*.

Keywords Gene regulation · Glutathione · Homoglutathione · *Medicago* · Nitric oxide

Abbreviations

γ-EC	Gamma-glutamylcysteine
γ-ECS	Gamma-glutamylcysteine synthetase
FeCN	Ferricyanide
GSH	Glutathione
GSHS	Glutathione synthetase
GSNO	Nitrosoglutathione
hGSH	Homoglutathione
hGSHS	Homoglutathione synthetase
JA	Jasmonic acid
NO	Nitric oxide
SNP	Sodium nitroprusside

Introduction

The thiol tripeptide glutathione (GSH; γ-glutamylcysteinylglycine) is the major low molecular weight thiol present in plant species. It is involved in the storage and the transport of reduced sulphur (Kopriva and Rennenberg 2004), in the detoxification of xenobiotics via glutathione-*S*-transferase (Dixon et al. 2002), in the protection against heavy metals as a precursor in the synthesis of phytochelatins (Cobbett 2000), in the scavenging of active oxygen species by the ascorbate-glutathione cycle and in the regulation of the redox homeostasis of the cell (Noctor and Foyer 1998). Homologs of GSH have been detected in different plant

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families (Price 1957; Klapheck et al. 1992; Meuwly et al. 1993). All these compounds share an equivalent chemical structure, which points to similar functions in plants. Legumes are one of the plants in which two different low molecular weight thiols coexist in the same plant. Homoglutathione (hGSH) is present instead of, or in addition to GSH (Klapheck 1988; Matamoros et al. 1999). The relative amount of GSH and hGSH has been shown to vary considerably between different organs (Frendo et al. 1999; Matamoros et al. 1999).

The synthesis of the GSH and hGSH is an ATP-dependent two-step reaction. In a first common step catalysed by the γ-glutamylcysteine synthetase (γ-ECS; EC 6.3.2.2.), γ-glutamylcysteine (γ-EC) is produced from L-glutamic acid and L-cysteine. Thus, the synthesis of GSH/hGSH is linked to nitrogen assimilation via the glutamic acid and to sulphur metabolism through the cysteine. In a second step, glutathione synthetase (GSHS; EC 6.3.2.3) catalyses the formation of GSH by adding glycine to the C-terminal carboxy group of γ-EC whereas homoglutathione synthetase (hGSHS; EC 6.3.2.23) catalyses the formation of hGSH by adding β-alanine to the γ-EC.

Nitric oxide (NO) is a gaseous bioactive molecule that participates in the regulation of a large range of developmental and pathophysiological processes in animals (Mayer and Hemmens 1997). During the last decade, NO also emerged as a pivotal regulator of plant physiology and has been associated with processes as diverse as iron homeostasis control, flowering, and abiotic and biotic stress responses (Delledonne et al. 1998; Durner et al. 1998; Desikan et al. 2002; Graziano et al. 2002). At the molecular level, NO has been implicated in the regulation of gene expression (for a review, Grun et al. 2006). For instance, the induction of the gene coding the iron-storage protein ferritin upon iron treatment is mediated by NO (Murgia et al. 2002). Recent transcriptomic studies indicate that the genes regulated by NO sustain a large diversity of cellular functions, in accordance with the pleiotropic role of this molecule in plant physiology (Huang et al. 2002; Polverari et al. 2003; Parani et al. 2004). NO can also directly interact with cellular constituents such as reactive oxygen species (ROS). It is noteworthy that the ROS/NO interaction may be cytotoxic or protective depending on the relative ROS/NO concentrations (Delledonne et al. 2001; Beligni et al. 2002; Orozco-Cardenas and Ryan 2002).

Several studies have evidenced that the GSH biosynthetic pathway is stimulated in response to NO in animal cells and yeast (Kuo and Abe 1996; Moellering et al. 1998; Kim et al. 2004). In these reports, the increase of GSH synthesis was attributed to an enhanced expression of the rate-limiting enzyme γ-ECS. Using *Medicago truncatula* as a model, we now show that NO can also regulate GSH synthesis in plants, by stimulating *γ-ecs* and *gshs* gene expression. Moreover, our data indicate a differential regulation of *gshs* and *hgshs* in response to NO.

Materials and methods

Plant-growth conditions and treatments

Medicago truncatula ecotype Jemalong was used for all the experiments (kindly provided by Dr. G. Duc, URLEG, 21110 Bretenières, France). Sterilized seedlings germinated for 48 h on 0.5% agarose were planted in sand watered with modified Farhaeus medium (Boisson-dernier et al. 2001). Plants were grown for 4 weeks on sand watered with nutritive medium in a growth chamber with a day/night temperature of 22°C and with a photoperiod of 16 h. After 4 weeks, plants were transferred in modified Farhaeus liquid medium containing 1 mM sodium nitroprusside (SNP), 1 mM potassium ferricyanide (FeCN), 0.5 mM nitrosoglutathione (GSNO) or 0.5 mM glutathione (GSH) under light condition. For the buthionine sulfoximine (BSO) treatment, plants were pre-treated for 6 h in modified Farhaeus liquid medium containing 1 mM BSO. SNP and FeCN were added to the medium subsequently. No stress symptoms were visible during the time course of our experiments.

RNA analysis

RNAs were extracted from *M. truncatula* roots using Trizol according to manufacturer procedure protocol (Gibco BRL Life Technologies). RNA samples (10 μg) were fractionated on 1.4% formaldehyde–agarose gels, transferred onto Hybond N membrane (Amersham) and hybridised according to standard protocols (Sambrook et al. 1989). Blots were hybridised with γ-ECS, GSHS and hGSHS cDNAs, and ribosomal RNA hybridisation served as RNA loading control. Phosphoimager quantification of signal intensities corresponding to the expression of *γ-ecs*, *gshs* and *hgshs* was performed with Fujifilm Bio-Imaging Analyser BAS-1000. The expression levels of *γ-ecs*, *gshs* and *hgshs* genes were standardised using the ribosomal RNA expression.

Determination of GSH and hGSH

Thiols were extracted with hydrochloric acid, derivatized with monobromobimane and quantified after

separation on reverse phase HPLC as described by Fahey and Newton (1987). Commercial GSH (Sigma) and hGSH synthesized by Neosystem (Strasbourg, France) were used as standards.

Statistical analyses

All the data presented for thiol concentrations are given as means with the standard deviation of three independent experiments. The significance of the results compared to the control was tested using the Student's *t* test.

Results

To test whether NO regulates glutathione synthesis pathway, we studied the expression of the genes involved in GSH and hGSH synthesis in *M. truncatula* after treatment with the NO donors SNP and GSNO, which release NO^+ and $NO^\cdot$, respectively (Stamler et al. 1992; Hogg 2000). As controls, plants were treated with the SNP degradation product potassium ferricyanide (FeCN), which is unable to release NO, and with GSH, which is produced after NO release from GSNO. The accumulation of γ-*ecs, gshs* and *hgshs* mRNA after treatment with SNP or FeCN was monitored by Northern-blot analysis (Fig. 1a). The level of γ-*ecs* and *gshs* transcripts was increased three and eightfold, respectively, following SNP treatment, with a concomitant maximum of accumulation 6 h after NO donor application. FeCN also induced an accumulation of γ-*ecs* and *gshs* transcripts but to a much lower extent (1.3-fold and 4-fold, respectively). In contrast, there was a diminution of the accumulation of *hgshs* mRNA after both the SNP treatment and the control-FeCN treatment. Taken together, these results indicate that there is a similar regulation of γ-*ecs* and *gshs* genes, with an induction of both gene expressions after SNP treatment. In contrast, *hgshs* gene expression does not appear to be NO regulated.

A similar analysis of the γ-*ecs*, *gshs* and *hgshs* gene expression was performed using GSNO, a NO donor structurally unrelated to SNP. The level of γ-*ecs* and *gshs* transcripts was increased up to 2.5- and 6-fold, respectively, following GSNO treatment with a maximum of accumulation 12 h after the NO donor application (Fig. 1b). No significant difference was observed when plants were either treated with GSH or modified Farheus medium alone. In contrast, *hgshs* transcript amounts decreased twofold following GSNO application compared to the plants either treated with GSH or modified Farheus medium alone. Taken together,

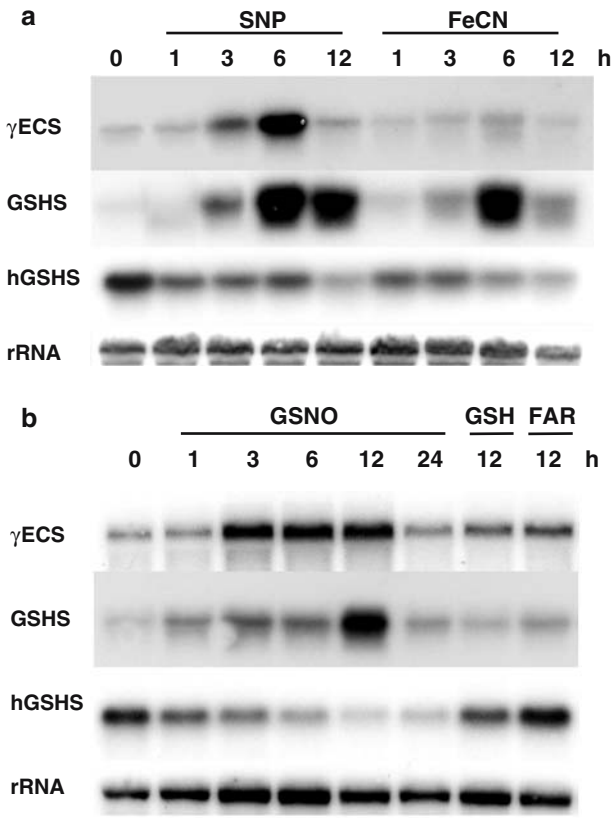

Fig. 1 Analysis of the expression of *γecs, gshs* and *hgshs* in SNP, FeCN, GSNO and GSH-treated roots. **a** Plants were treated with modified Farhaeus medium containing 1 mM SNP and 1 mM FeCN. Root tissue was collected at set times after treatment. RNA gel blots were successively probed with *γ-ecs, gshs* and *hgshs* cDNAs and ribosomal RNA. Each experiment has been repeated twice. **b** Plants were treated with modified Farhaeus medium alone (*FAR*) or containing 0.5 mM GSNO (*GSNO*) and 0.5 mM GSH (*GSH*). Root tissue was collected at set times after treatement. RNA gel blots were successively probed with *γ-ecs, gshs* and *hgshs* cDNAs and ribosomal RNA. Each experiment has been repeated twice

these results showed that NO, generated either by SNP or GSNO application, was able to induce the expression of γ-*ecs* and *gshs* genes. In contrast, no effect or even a negative regulation of *hgshs* gene expression after NO treatment was observed.

To test whether the induction of γ-*ecs* and *gshs* genes was followed by the accumulation of GSH in plants, analysis of GSH and hGSH content was performed in SNP (Fig. 2a) and GSNO treated roots (data not shown). A significant threefold increase of GSH content over control was observed 24 h after SNP application. A similar increase of hGSH content was also detected. To test whether GSH and hGSH accumulation was linked to the γ-*ecs* gene induction, plants were treated with buthionine sulfoximine (BSO), a specific inhibitor of γ-ECS (Fig. 2b). Treatment with BSO reduced the accumulation of GSH and hGSH observed in SNP-treated plants

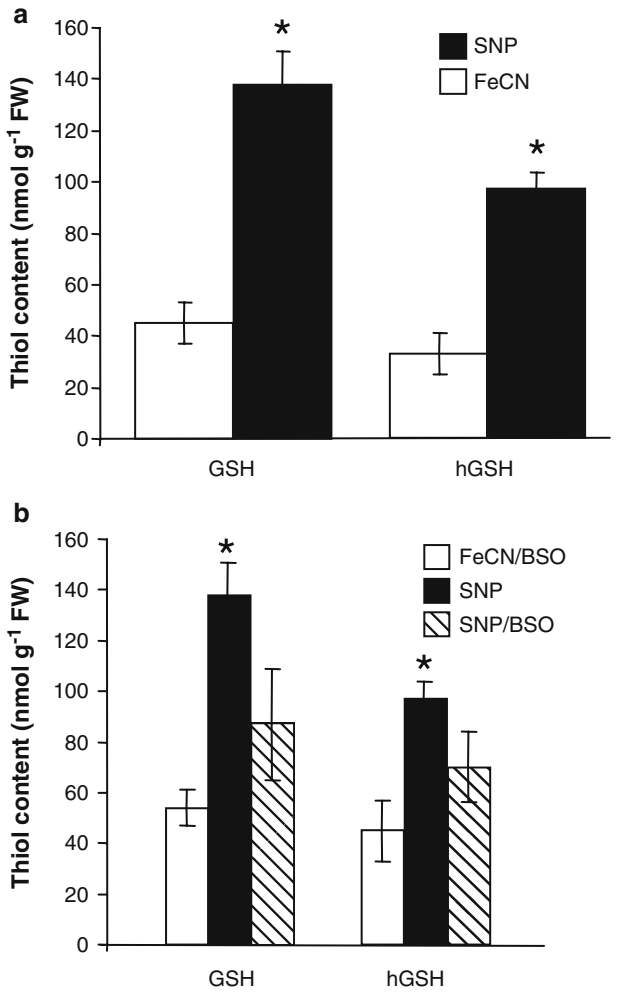

Fig. 2 Analysis of the GSH and hGSH content in SNP, FeCN and BSO-treated roots. **a** Plants were treated with modified Farhaeus medium containing 1 mM SNP (*SNP*), 1 mM FeCN (*FeCN*). Root tissue was collected 24 h after treatment. The GSH and hGSH total content was quantified by HPLC analysis. Each experiment has been repeated three times. Data are presented as the mean ± SD of the results. * indicates a significant difference compared to the control ($P < 0.05$). **b** Plants were treated with modified Farhaeus medium containing both 1 mM FeCN and 1 mM BSO (*FeCN/BSO*), SNP (*SNP*) and both 1 mM SNP and 1 mM BSO (*SNP/BSO*). Root tissue was collected 24 h after treatment. The GSH and hGSH total content was quantified by HPLC analysis. Each experiment has been repeated three times. Data are presented as the mean ± SD of the results. * indicates a significant difference compared to the control ($P < 0.05$)

by 60 and 50%, respectively; this shows that the SNP-dependent thiol accumulation is linked, at least partially, to the γ-ECS activity.

As GSNO decay generated a massive artefactual production of GSH, we could not determine the endogenous GSH content following GSNO treatment. hGSH accumulation was not significantly modified over the time course of GSNO treatment (data not shown).

Taken together, these data indicate that there is a different regulation of GSH and hGSH synthesis pathway in response to NO in *M. truncatula*. Indeed, NO induced concomitantly the expression of γ-*ecs* and *gshs* genes, which was correlated to the accumulation of the end product GSH. In contrast, no significant effect was observed on *hgshs* expression and the accumulation of hGSH observed during the SNP treatment appeared to be linked to the augmentation of γ-ECS activity.

Discussion

In the present study, the effect of NO on the GSH/hGSH synthesis pathway was examined in roots of *M. truncatula*. Generation of NO was achieved by treatment of roots with SNP and GNSO, two different NO-donors with unrelated structures, which have been widely used to analyse gene expression in plants (Durner et al. 1998; Polverari et al. 2003; Murgia et al. 2004). Our data provide the first evidence that GSH synthesis is stimulated by NO in plants. This result is in contrast with those of de Pinto and colleagues (2002), who reported a decrease of GSH content in SNP-treated BY-2 tobacco cells, suggesting a different response between cell culture and roots. Nevertheless, a similar response was previously reported for fission yeast and animal cells (Kuo and Abe 1996; Moellering et al. 1998; Kim et al. 2004), and the present report extends the effect of NO on GSH synthesis pathway to plants. As for yeast and animals, NO triggered an increase of the endogenous GSH amount above control in *M. truncatula* roots through the stimulation of GSH synthesis gene transcript accumulation. Whereas only *γ-ecs* gene stimulation was tested for GSH accumulation upon NO treatment in yeast and animals (Kuo and Abe 1996; Kim et al. 2004), our results underline a concomitant up-regulation of γ-*ecs* and *gshs* gene transcription in *M. truncatula*.

A specific feature of legumes is the presence of second tripeptide thiol, e.g. hGSH, in addition to GSH. In *M. truncatula*, hGSH is only present in the underground organs, e.g. roots and nodules, whereas GSH is present in the whole plant (Frendo et al. 1999). Our results evidence that *hgshs* and *gshs* genes are regulated differently by NO. Indeed, SNP and GSNO treatments do not induce significantly or even repress the expression of the *hgshs* gene. Although a reduction in *hgshs* mRNA level is observed, it is not followed by a decrease of hGSH level, which even increased upon SNP treatment. This apparent discrepancy between the *hgshs* expression and hGSH accumulation may be explained by the higher accumulation of γ-EC consecutive to the

higher expression of the γ-*ecs*. Indeed, SNP-stimulated hGSH synthesis is strongly impaired when γ-EC synthesis is inhibited by BSO treatment. Moreover, the activity of hGSHS is three times higher than that of GSHS in *M. truncatula* roots (Frendo et al. 1999). This difference may also explain the accumulation of hGSH in presence of a higher level of γ-EC. Finally, we cannot exclude that the hGSHS half-life is high enough to prevent a decrease of hGSH content after the *hgshs* gene repression during the time course of the GSNO treatment. A similar differential expression of *gshs* and *hgshs* has been reported in response to heavy metals, which stimulated the expression of γ-*ecs* and *gshs* genes but not that of *hgshs* gene in *M. truncatula* roots (Harrison et al. 2003). These data, together with the different localisation of GSH and hGSH in legumes containing both compounds, raise the hypothesis that different biological functions may be preferentially sustained by these two low molecular weight thiols in these plants.

Although multiple factors including abiotic stresses, i.e. drought, salt and temperature stresses, or pathogenic interactions can affect the GSH/hGSH pool in plants, few conditions are known to modify the GSH biosynthesis pathway at the transcriptional level. To our knowledge, such regulation was only observed in response to heavy metals and jasmonic acid (JA) treatments (Xiang and Oliver 1998; Sasaki-Sekimoto et al. 2005). We showed that NO can also regulate GSH biosynthesis through the regulation of γ-*ecs* and *gshs* gene expression. The existence of cross talks between JA and NO signalling pathways, which are responsible for the expression of specific genes, has been reported (Huang et al. 2004; Wang and Wu 2005). It would therefore be of interest to investigate whether NO may act as a mediator in the regulation of GSH synthesis by JA, and possibly by heavy metals, or acts independently of these inducers.

The regulation of GSH synthesis by NO raises the question of the physiological roles that may be sustained by such a modulation. Several studies have evidenced the capacity of NO to counteract oxidative damages (Beligni and Lamattina 1999; Beligni et al. 2002; Wang and Wu 2005). Nevertheless, how such an anti-oxidative activity occurs is unclear. In addition to a direct reactive oxygen species scavenging activity and to the modulation of lipid peroxidation by lipoxygenase inhibition, NO may also protect cells against oxidative processes by stimulating GSH synthesis. This mechanism of protection has been proposed in animal cells (Kuo and Abe 1996; Moellering et al. 1998) and may be operative in plants as well. GSH may also play an important role in regulating NO bioactivity. Indeed, it can readily react with NO to form GSNO, which serves as a NO reservoir and a long-distance NO vector in mammals (Zhang and Hogg 2004). In regard of recent reports indicating the importance of nitrosothiols in controlling plant responses to pathogens (Feechan et al. 2005), the stimulation of GSH synthesis by NO may provide an important regulatory loop for NO bioactivity.

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References

Beligni MV, Lamattina L (1999) Nitric oxide protects against cellular damage produced by methylviologen herbicides in potato plants. Nitric Oxide 3:199–208

Beligni MV, Fath A, Bethke PC, Lamattina L, Jones RL (2002) Nitric oxide acts as an antioxidant and delays programmed cell death in barley aleurone layers. Plant Physiol 129:1642–1650

Boisson-dernier A, Chabaud M, Garcia F, Bécard G, Rosenberg C, Barker DG (2001) *Agrobacterium rhizogene*-transformed roots of *Medicago truncatula* for the study of nitrogen-fixing and endomycorrhizal symbiotic associations. Mol Plant Microbe Interact 14:695–700

Cobbett CS (2000) Phytochelatins and their roles in heavy metal detoxification. Plant Physiol 123:825–832

Delledonne M, Xia Y, Dixon RA, Lamb C (1998) Nitric oxide functions as a signal in plant disease resistance. Nature 394:585–588

Delledonne M, Zeier J, Marocco A, Lamb C (2001) Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. Proc Natl Acad Sci USA 98:13454–13459

de Pinto MC, Tommasi F, De Gara L (2002) Changes in the antioxidant systems as part of the signaling pathway responsible for the programmed cell death activated by nitric oxide and reactive oxygen species in tobacco Bright-Yellow 2 cells. Plant Physiol 130:698–708

Desikan R, Griffiths R, Hancock J, Neill S (2002) A new role for an old enzyme: nitrate reductase-mediated nitric oxide generation is required for abscisic acid-induced stomatal closure in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 99:16314–16318

Dixon DP, Lapthorn A, Edwards R (2002) Plant glutathione transferases. Genome Biol 3:3004.1–3004.10

Durner J, Wendehenne D, Klessig DF (1998) Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose. Proc Natl Acad Sci USA 95:10328–10333

Feechan A, Kwon E, Yun BW, Wang Y, Pallas JA, Loake GJ (2005) A central role for *S*-nitrosothiols in plant disease resistance. Proc Natl Acad Sci USA 102:8054–8059

Frendo P, Gallesi D, Turnbull R, Van de Sype G, Hérouart D, Puppo A (1999) Localisation of glutathione and homoglutathione in *Medicago truncatula* is correlated to a differential expression of genes involved in their synthesis. Plant J 17:215–219

Graziano M, Beligni MV, Lamattina L (2002) Nitric oxide improves internal iron availability in plants. Plant Physiol 130:1852–1859

Grun S, Lindermayr C, Sell S, Durner J (2006) Nitric oxide and gene regulation in plants. J Exp Bot 57:507–516

Harrison J, Puppo A, Frendo P (2003) The synthesis and the roles of glutathione and homoglutathione in legumes. Adv Plant Physiol 6:385–412

Hogg N (2000) Biological chemistry and clinical potential of S-nitrosothiols. Free Radic Biol Med 28:1478–1486

Huang X, von Rad U, Durner J (2002) Nitric oxide induces transcriptional activation of the nitric oxide-tolerant alternative oxidase in *Arabidopsis* suspension cells. Planta 215:914–923

Huang X, Stettmaier K, Michel C, Hutzler P, Mueller MJ, Durner J (2004) Nitric oxide is induced by wounding and influences jasmonic acid signaling in *Arabidopsis thaliana*. Planta 218:938–946

Kim JM, Kim H, Kwon SB, Lee SY, Chung SC, Jeong DW, Min BM (2004) Intracellular glutathione status regulates mouse bone marrow monocyte-derived macrophage differentiation and phagocytic activity. Biochem Biophys Res Commun 325:101–108

Klapheck S (1988) Homoglutathione: isolation, quantification and occurrence in legumes. Physiol Plant 74:727–732

Klapheck S, Chrost B, Starke J, Zimmermann H (1992) γ-glutamyl-cysteinylserine—a new homologue of glutathione in plants of the family Poaceae. Bot Acta 105:174–179

Kopriva S, Rennenberg H (2004) Control of sulphate assimilation and glutathione synthesis: interaction with N and C metabolism. J Exp Bot 55:1831–1842

Kuo PC, Abe KY (1996) Nitric oxide-associated regulation of hepatocyte glutathione synthesis is a guanylyl cyclase-independent event. Surgery 120:309–314

Matamoros MA, Baird LM, Escuredo PR, Dalton DA, Minchin FR, Iturbe-Ormaetxe I, Rubio MC, Moran JF, Gordon AJ, Becana M (1999) Stress induced legume root nodule senescence. Physiological, biochemical and structural alterations. Plant Physiol 121:97–112

Mayer B, Hemmens B (1997) Biosynthesis and action of nitric oxide in mammalian cells. Trends Biochem Sci 22:477–481

Meuwly P, Thibault P, Rauser WE (1993) γ-glutamylcysteinylglutamic acid—a new homologue of glutathione in maize seedlings exposed to cadmium. FEBS Lett 336:472–476

Moellering D, McAndrew J, Patel RP, Cornwell T, Lincoln T, Cao X, Messina JL, Forman HJ, Jo H, Darley-Usmar VM (1998) Nitric oxide-dependent induction of glutathione synthesis through increased expression of gamma-glutamylcysteine synthetase. Arch Biochem Biophys 358:74–82

Murgia I, Delledonne M, Soave C (2002) Nitric oxide mediates iron-induced ferritin accumulation in *Arabidopsis*. Plant J 30:521–528

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

Noctor G, Foyer C (1998) Ascorbate and glutathione: keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol 49:249–279

Orozco-Cardenas ML, Ryan CA (2002) Nitric oxide negatively modulates wound signaling in tomato plants. Plant Physiol 130:487–493

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

Polverari A, Molesini B, Pezzotti M, Buonaurio R, Marte M, Delledonne M (2003) Nitric oxide-mediated transcriptional changes in *Arabidopsis thaliana.* Mol Plant Microbe Interact 16:1094–1105

Price C (1957) A new thiol in legumes. Nature 180:148–149

Sambrook J, Fritsch EF, Maniatis TA (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York

Sasaki-Sekimoto Y, Taki N, Obayashi T, Aono M, Matsumoto F, Sakurai N, Suzuki H, Hirai MY, Noji M, Saito K, Masuda T, Takamiya K, Shibata D, Ohta H (2005) Coordinated activation of metabolic pathways for antioxidants and defence compounds by jasmonates and their roles in stress tolerance in *Arabidopsis*. Plant J 44:653–668

Stamler JS, Singel DJ, Loscalzo J (1992) Biochemistry of nitric oxide and its redox-activated forms. Science 258:1898–1902

Wang JW, Wu JY (2005) Nitric oxide is involved in methyl jasmonate-induced defense responses and secondary metabolism activities of *Taxus* cells. Plant Cell Physiol 46:923–930

Xiang C, Oliver DJ (1998) Glutathione metabolic genes coordinately respond to heavy metals and jasmonic acid in *Arabidopsis*. Plant Cell 10:1539–1550

Zhang Y, Hogg N (2004) The mechanism of transmembrane *S*-nitrosothiol transport. Proc Natl Acad Sci USA 101:7891–7896