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Up-Regulation of Heme Oxygenase by Nitric Oxide and Effect of Carbon Monoxide on Soybean Plants Subjected to Salinity

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1. Introduction

Reactive oxygen species (ROS) are generated in small amounts in the normal metabolism of the cells and in increased amounts under many conditions of altered cell physiology; they are responsible for many kinds of cell injuries (Sies 1993) and have been shown to induce a significant reprogramming of gene expression (Colburn 1992).

Salt stress is one of the most important abiotic stresses that adversely affects soybean growth and causes significant crop loss worldwide. Salinity has always been considered a serious constraint on agricultural productivity (Hay & Porter 2006) and affects plant's physiology. Salt stress is a complex phenomenon that involves morphological and developmental changes. Two major components have been identified in this insult, osmotic stress and ion toxicity (Darwish et al. 2009). Higher plants have multiple protective mechanisms against salt stress including ion homeostasis, osmolyte biosynthesis, ROS scavenging, water transport, and transducers of long-distance response coordination. It is generally accepted that many stresses, including salinity, induce an overproduction of ROS, such as H_2O_2 , $O_2^{\bullet-}$, and $HO\cdot$, and these species are thought to be responsible for the oxidative damage associated with plant stress (Zilli et al. 2009). To counteract the toxicity of ROS, defense systems that scavenge cellular ROS have been developed in plants to cope with oxidative stress via the non-enzymatic and enzymatic systems (Demiral & Turkan 2005; Mandhania et al. 2006).

Nitric oxide (NO) acts as a signaling molecule and mediates multiple physiological processes in plants (Leitner et al. 2009). In addition, it has been implicated in responses to biotic and abiotic stresses, such as disease resistance, salinity, drought, heat stress, among others (Beligni & Lamattina 1999; Romero-Puerta et al. 2004; Corpas et al. 2009). There are several sources of NO in plants, but mainly it can be enzymatically produced by nitrate reductase and nitric oxide synthase-like enzymes (Wilson et al. 2008 and Corpas et al. 2009). NO is a reactive nitrogen species and, depending on its concentration, it produces either protective or toxic effects. A low dose of NO modulates superoxide anion formation and inhibits lipid peroxidation, resulting in an antioxidant function during stress (Boveris et al. 2000 and Santa Cruz et al. 2010). Moreover, microarray studies have shown that NO induces a large number of genes at transcriptional level, among them those of antioxidant enzymes (Parani et al. 2004). It has also been reported that Nitric oxide gives rise to signaling pathways mediating

responses of specific genes to ultraviolet-B (UV-B) radiation, such as chalcone synthase and phenylalanine ammonia lyase (Mackerness et al. 2001). However, information about the role that NO plays in regulation of antioxidant enzymes to counteract salt-induced oxidative stress is rather limited.

Nitric oxide is believed to act as a signal molecule mediating responses to both biotic and abiotic stresses in plants (reviewed in Xuan et al. 2010 and Nürnberger & Scheel 2001) and its presence has been shown to induce seed germination (Liu et al. 2010), to affect growth and development of plant tissue (Beligni & Lamatina 2001, to increase iron homeostasis (Martin et al. 2009), to regulate plant maturation and senescence (Yaacov et al. 1998 and Jasid et al. 2009) to mediate abscisic acid-induced stomatal closing (Garcia-Mata & Lamattina, 2007). Recently, a few studies suggested that NO can play a role in protecting plants from oxidative stresses (Shantel et al. 2008) and NO-donor treatment protected plants from damage by increasing the activity of antioxidative enzymes.

Heme oxygenase catalyzes the oxidative degradation of heme and has well-known antioxidant properties in mammals by mean of its products biliverdin IX α and carbon monoxide (CO) (Kikuchi et al. 2005). One of the three known mammalian isoforms, heme oxygenase-1 (HO-1), is induced in animal tissues by many factors including its own substrate heme, heavy metals, UV-A radiation among others (Tomaro & Batlle 2002). While earlier studies pointed to plant HO as a source of phytochrome chromophore (Terry et al. 2002), more recent works showed that HO synthesis increases in soybean plants subjected to oxidative stress conferring resistance to a subsequent insult (Noriega et al. 2004; Balestrasse et al. 2005). Moreover, we have recently demonstrated that ROS are involved in HO-1 up-regulation in soybean leaves subjected to UV-B radiation (Yannarelli et al. 2006 and Santa Cruz et al. 2010). We hypothesized that NO may also participate in this process, as it regulates the oxidative status and mediates other UV-B responses.

The aim of the present study was to investigate whether NO or CO could protect soybean against salt-induced oxidative stress through the modulation of HO activity. Soybean plants were subjected to salt stress after pre-treatments with different concentrations of sodium nitroprussiate (SNP), a well-characterized NO-donor or CO. Overall, our results indicate that in soybean plants NO is involved in the signaling pathway leading to HO-1 up-regulation under salinity, and that a balance between NO and ROS is important to trigger the antioxidant response against oxidative stress. On the other hand pretreatment with CO did not provoke any change.

2. Materials and methods

2.1 Plant material and treatments

Surface sterilized soybean seeds (*Glycine max.* L.) (A6445RG) were germinated for 10 days in plastic pots containing vermiculite in controlled environmental chambers, with a photoperiod of 16 h, photon flux density of 175 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and a day/night regime of 25/20°C. Afterwards, they were pretreated hydroponically with different sodium nitroprussiate concentrations (250-750 μM) for 72 h and then with NaCl (200 mM) for 48 h.

Carbon monoxide was generated from H_2SO_4 and formic acid (HCOOH). Stock solution was prepared by bubbling CO in a Hoagland solution for 40 min and was immediately diluted (50%) to perform analysis.

Plants were then harvested. When the effect of Zn-protoporphyrin IX (ZnPPIX) was investigated, roots were pretreated with 22 μM ZnPPIX during 4 h before addition of NaCl.

Controls were incubated in buffer. For fresh weight determination, plants were filtered, washed three times with distilled water, kept on filter paper for a few minutes to remove of excess liquid and weighed. Three different experiments were performed, with three replicated measurements for each parameter assayed

2.2 Thiobarbituric acid reactive substances (TBARS) determination

Lipid peroxidation was measured as the amount of TBARS determined by the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). Fresh control and treated roots (0.3 g) were homogenized in 3ml of 20% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at $3,500 \times g$ for 20 min. To 1 ml of the aliquot of the supernatant, 1ml of 20% TCA containing 0.5% (w/v) TBA and 100 ml 4% butylated hydroxytoluene (BHT) in ethanol were added. The mixture was heated at 95°C for 30min and then quickly cooled on ice. The contents were centrifuged at $10,000 \times g$ for 15 min and the absorbance was measured at 532 nm. Value for non-specific absorption at 600 nm was subtracted. The concentration of TBARS was calculated using an extinction coefficient of $155 \text{ mM}^{-1}\text{cm}^{-1}$

2.3 Heme oxygenase preparation and assay

Roots (0.3 g) were homogenized in a Potter-Elvehjem homogenizer using 4 vol. of ice-cold 0.25M sucrose solution containing 1mM phenylmethyl sulfonyl fluoride, 0.2mM EDTA and 50mM potassium phosphate buffer (pH 7.4). Homogenates were centrifuged at $20,000 \times g$ for 20min and supernatant fractions were used for activity determination. Heme oxygenase activity was determined as previously described with minor modifications (Muramoto et al. 2002). The standard incubation mixture in a final volume of 500ml contained 10mmol potassium phosphate buffer (pH 7.4), 60 nmol NADPH, 250ml HO (0.5mg protein), and 200 nmol hemin. Incubations were carried out at 37°C during 60min. Activity was determined by measuring biliverdin formation, which was calculated using the absorbance change at 650 nm employing an ϵ value of $6.25 \text{ mM}^{-1}\text{cm}^{-1}$ (ϵ_{max} 650 nm)

2.4 Glutathione determination

Non-protein thiols were extracted by homogenizing 0.3 g of roots in 3.0 ml of 0.1 N HCl (pH 2.0), and 1 g PVP. After centrifugation at $10,000 \times g$ for 30 min at 4°C , the supernatants were used for analysis. Total glutathione (GSH plus GSSG) was determined in the homogenates spectrophotometrically at 412 nm, after precipitation with 0.1 N HCl, using yeast-glutathione reductase, 5,5' dithio-bis-(2-nitrobenzoic acid) (DTNB) and NADPH. GSSG was determined by the same method in the presence of 2-vinylpyridine and GSH content was calculated from the difference between total glutathione and GSSG (Anderson, 1985).

2.5 Classical antioxidant enzymes

Extracts for determination of catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) activities were prepared from 0.3 g of roots homogenized under ice-cold conditions in 3 ml of extraction buffer, containing 50 mM phosphate buffer (pH 7.4), 1 mM EDTA, 1 g PVP, and 0.5% (v/v) Triton X-100 at 4°C . The homogenates were centrifuged at $10,000 \times g$ for 20 min and the supernatant fraction was used for the assays.

CAT activity was determined in the homogenates by measuring the decrease in absorption at 240 nm in a reaction medium containing 50 mM potassium phosphate buffer (pH 7.2) and 2 mM H_2O_2 . The pseudo-first order reaction constant ($k' = k[\text{CAT}]$) of the decrease in H_2O_2

absorption was determined and the catalase content in pmol mg⁻¹ protein was calculated using $k = 4.7 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$.

APX activity was measured immediately in fresh extracts and was assayed as described by Nakano and Asada (1981), using a reaction mixture (1 ml) containing 50 mM K-phosphate buffer (pH 7.0), 0.1 mM H₂O₂, 0.5 mM Na-Ascorbate and 0.1 mM EDTA. The hydrogen peroxide-dependent oxidation of Ascorbate was followed by a decrease in the absorbance at 290 nm (ϵ : 2.8 mM⁻¹ cm⁻¹). One unit of APX forms 1 μmol of ascorbate oxidized per minute under the assay conditions.

GR activity was measured by following the decrease in absorbance at 340 nm due to NADPH oxidation. The reaction mixture contained tissue extract, 1 mM EDTA, 0.5 mM GSSG, 0.15 mM NADPH and 50 mM Tris-HCl buffer (pH 7.5) and 3 mM MgCl₂ (Schaedle and Bassham 1977).

2.6 Histochemical analysis

In order to analyze H₂O₂ generation roots were excised and immersed in a 1% solution of 3,3'-Diaminobenzidine (DAB) in Tris-HCl buffer (pH 6.5), vacuum-infiltrated for 5 min and then incubated at room temperature for 16 h in the absence of light. Roots were illuminated until appearance of brown colors characteristic of the reaction of DAB with H₂O₂.

In the same way to show O₂⁻ production roots were excised and immersed in a 0.1% solution of NBT in K-phosphate buffer (pH 6.4), containing 10 mM Na-azide, and were vacuum-infiltrated for 5 min and illuminated until appearance of dark spots, characteristic of blue formazan precipitate.

2.7 Isolation of RNA and RT-PCR analysis

Total RNA was extracted from soybean roots by using the Trizol reagent (Gibco BRL). Four micrograms of total RNA were treated with RNase-free DNase I (Promega, CA, USA) and then 1.0 μg was reversed transcribed into cDNA using random hexamers and M-MLV Superscript II RT (Invitrogen, CA, USA). PCR reactions were carried out using *Glycine max* HO-1 and 18S specific primers, as previously described (Yannarelli and others, 2006). The PCR profile was set at 94°C for 1 min and then 29 cycles at 94°C for 0.5 min, 54°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 7 min. Each primer set was amplified using an optimized number of PCR cycles to ensure the linearity requirement for semi-quantitative RT-PCR analysis. The amplified transcripts were visualized on 1.5% agarose gels with the use of ethidium bromide. Gels were then scanned (Fotodyne Incorporated, WI, USA) and analyzed using Gel-Pro Analyzer 3.1 software (Media Cybernetics, MD, USA).

2.8 Protein determination

Protein concentration was evaluated by the method of Bradford (1976), using bovine serum albumin as a standard.

2.9 Statistics

Values in the text, figures and tables indicate mean values \pm SEM. Differences among treatments were analyzed by one-way ANOVA, taking $p < 0.05$ as significant according to Tukey's multiple range test.

3. Results

3.1 Growth parameter

Experiments were carried out in the presence of different SNP concentrations ranging from 200 to 750 μM . Root length was measured as a parameter to assess the optimal condition. Figure 1 shows that 250 μM SNP brought about a 45% increase in root length, whereas a diminution was observed under the other concentrations. Depending on its dose, NO can promote or inhibit root growth. According to these results, 250 μM SNP was chosen as the concentration to be used in pretreatment.

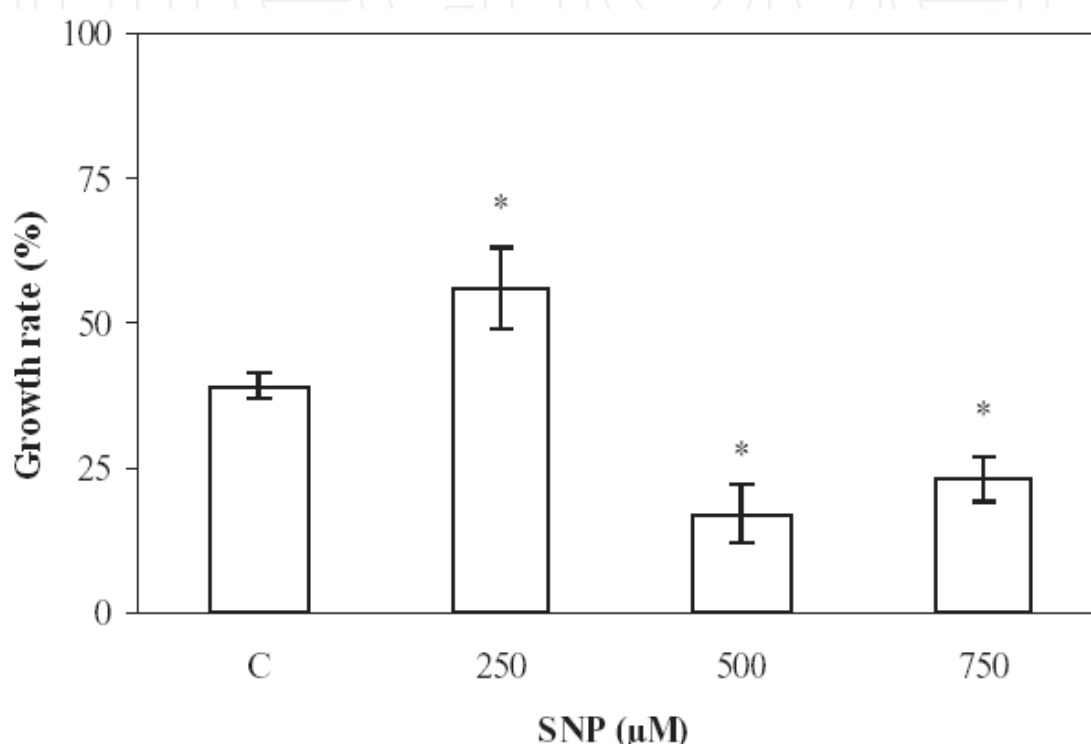


Fig. 1. Effect of different SNP concentrations on root growth. * Significant difference ($p < 0.05$) according to Tukey's test.

3.2 Lipid peroxidation

Increment in TBARS is a good reflection of oxidative damage to membrane lipids and other vital molecules such as proteins, DNA and RNA. Figure 2 shows that TBARS levels increased 75% respect to controls under salt treatment which is in agreement with results of other studies (Deng et al. 2010).

To complete this analysis, the effect of SNP pre-treatment was evaluated. Figure 2 indicates that in this case, membrane damage was more moderated, as indicated by a 14% augmentation respect to controls. Treatment with SNP alone did not show any difference respect to controls.

3.3 Glutathione content

GSH is a leading substrate for enzymatic antioxidant functions and it is also a known radical scavenger. Previous reports from our laboratory demonstrated that oxidative stress induces the formation of oxidant species and therefore affects GSH content in soybean plants

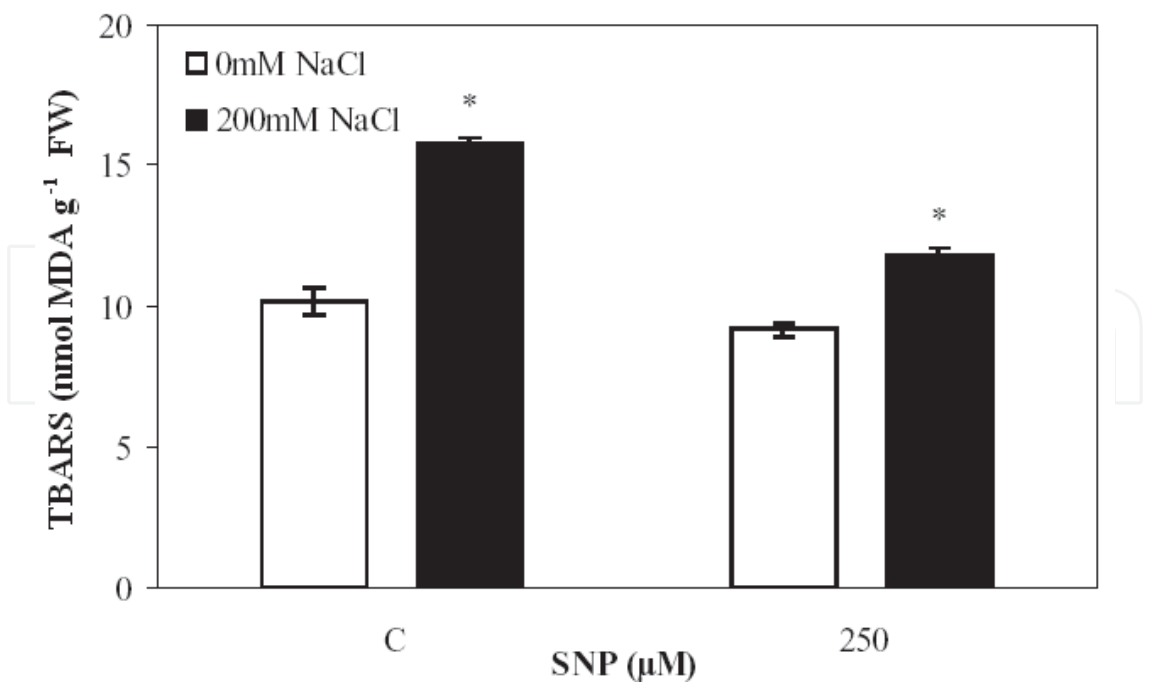


Fig. 2. Effect of salinity on TBARS formation and SNP regulation. * Significant difference (p<0.05) according to Tukey test.

(Balestrasse et al. 2001 and Noriega et al. 2004). Surprisingly, data in Figure 3 show that GSH concentration in soybean roots treated with NaCl was enhanced 3.5-fold respect to controls. Pre-treatment with SNP brought about a 4-fold augmentation respect to controls. Moreover, SNP alone provoked a 2-fold increase respect to controls.

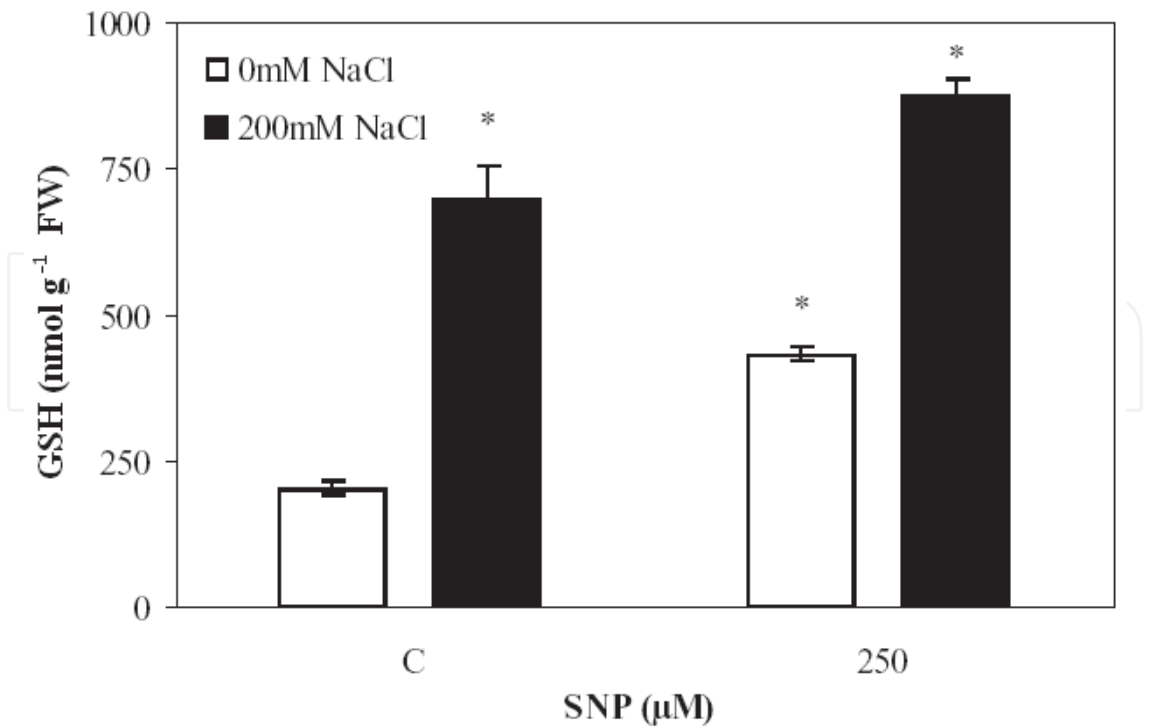


Fig. 3. Effect of salinity on GSH levels and SNP regulation. * Significant difference (p<0.05) according to Tukey test.

3.4 H₂O₂ and O₂^{•-} localization *in situ*

Accumulation of H₂O₂ and O₂^{•-} were also evaluated *in situ* by histochemical methods as shown in Figure 4a NaCl produced 32% H₂O₂ spots area versus total root area, while pretreatment with 250 μM SNP prevented this effect and spot area was similar to controls (Figure 4a). Data in Figure 4b showed that roots treated with NaCl produced 41% O₂^{•-} spots area versus total root area. Pretreatments with 250 μM SNP completely prevented the O₂^{•-} production induced by NaCl.

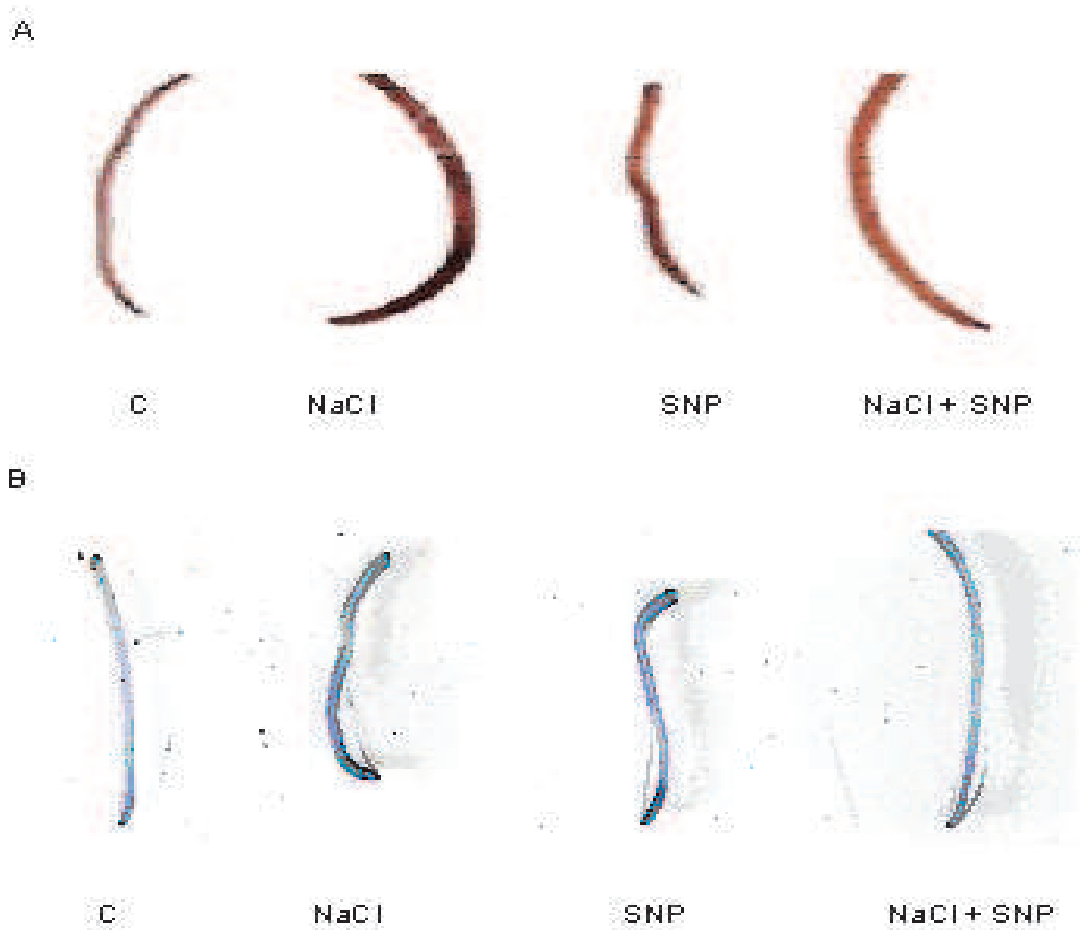


Fig. 4. Histochemical detection of H₂O₂ (A) and O₂^{•-} (B) in soybean roots. Experiments were performed as described in Materials and Methods. Pictures are representative of three different experiments with three replicated measurements for each treatment.

3.5 Effect of NO on antioxidant enzyme activities

We also investigated whether NO can modulate the activities of classical antioxidant enzymes such as CAT and APX. These are the main H₂O₂-scavenging enzymes that control ROS-mediated responses under biotic and abiotic stresses (Mittler 2002). CAT and APX activities were significantly affected by NaCl (Table 1). They were increased by 47% and 33% in NaCl-treated plants compared to controls, respectively. Moreover, CAT activity significantly augmented up to 24% with respect to controls of SNP-treated plants, whereas APX only showed a mild increase (19%). Heme oxygenase behavior was similar to that found for CAT (Table 1).

Treatment	HO-1 (U/mg protein)	CAT (pmol/mg protein)	APX (U/mg protein)
Control	0.065 ± 0.001 ^a	120 ± 12 ^a	0.0040 ± 0.0010 ^a
NaCl	0.073 ± 0.001 ^b	176 ± 9 ^b	0.0053± 0.0012 ^a
SNP	0.079 ± 0.002 ^c	149± 2 ^c	0.0047 ± 0.0010 ^a
SNP+ NaCl	0.083 ± 0.004 ^c	138 ± 14 ^c	0.0050 ±0.0010 ^a

Table 1. Antioxidant enzyme activities in soybean roots subjected to 200mM NaCl and 250 μM SNP pretreatment. Enzymatic activities were assayed as described in Materials and Methods. Different letters within columns indicate significant differences (*P* < 0.05) according to Tukey’s multiple range test.

3.6 Heme oxygenase-1 activity and gene expression

Previous findings from our group demonstrated the protective role that HO-1 plays against oxidative stress in soybean plants (Noriega et al. 2004 and Balestrasse et al. 2005). Figure 5 indicates that salt stress caused HO-1 mRNA induction (13%, respect to controls). This enhancement is positively correlated with enzyme activity (Table 1). Pretreatment with 250 μM SNP brought about an augmentation of gene expression in control plants (21%), as well as salt treated plants (27%) (Figure 5). Once again, this behavior was also found when enzyme activity was determinated (Table 1) .These results indicate on one hand, that NO

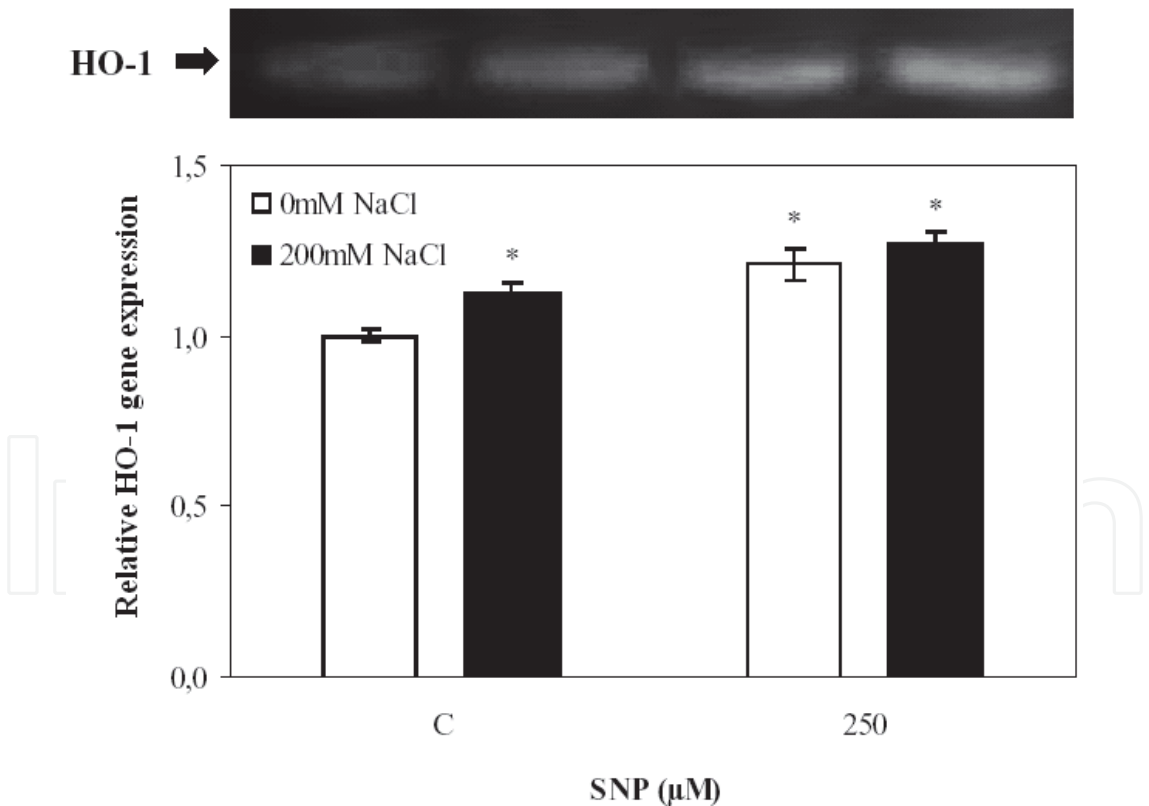


Fig. 5. HO-1 mRNA expression was analyzed by semi-quantitative RT-PCR as described in Materials and Methods. The 18S amplification band is shown to confirm equal loading of RNA and RT efficiency. Relative HO-1 transcript expression taking control as 1 U. Data are means of three independent experiments and bars indicate SE. *Significant differences (*P* < 0.05 according to Tukey test).

induces HO-1 more efficiently than NaCl, and on the other hand, both compounds have a synergic effect on this induction. To asses whether HO-1 is involved in the protection against NaCl exerted by NO, experiments were carried out in plants treated with ZnPPIX, a well known irreversible HO-1 inhibitor. Plants with inhibited HO-1 activity can not cope with NaCl insult (data not shown). We can assume that protection exerted by SNP may be due to the augmentation of the activity of this antioxidant enzyme.

3.7 Effect of NO and CO

3.7.1 Glutathione content

As already stated, there is a positive relationship between NO content and GSH levels (Figure 3). This result prompted us to investigate whether HO is involved in the regulation of this tripeptide. To fulfill this purpose, experiments were carried out in plants treated with ZnPPIX and then subjected to NO (HO inducer) or CO (HO reaction product) for 48 h before salt stress. Afterwards, GSH content (Figure 6) as well as HO-1 gene expression (Figure 8) was determinated.

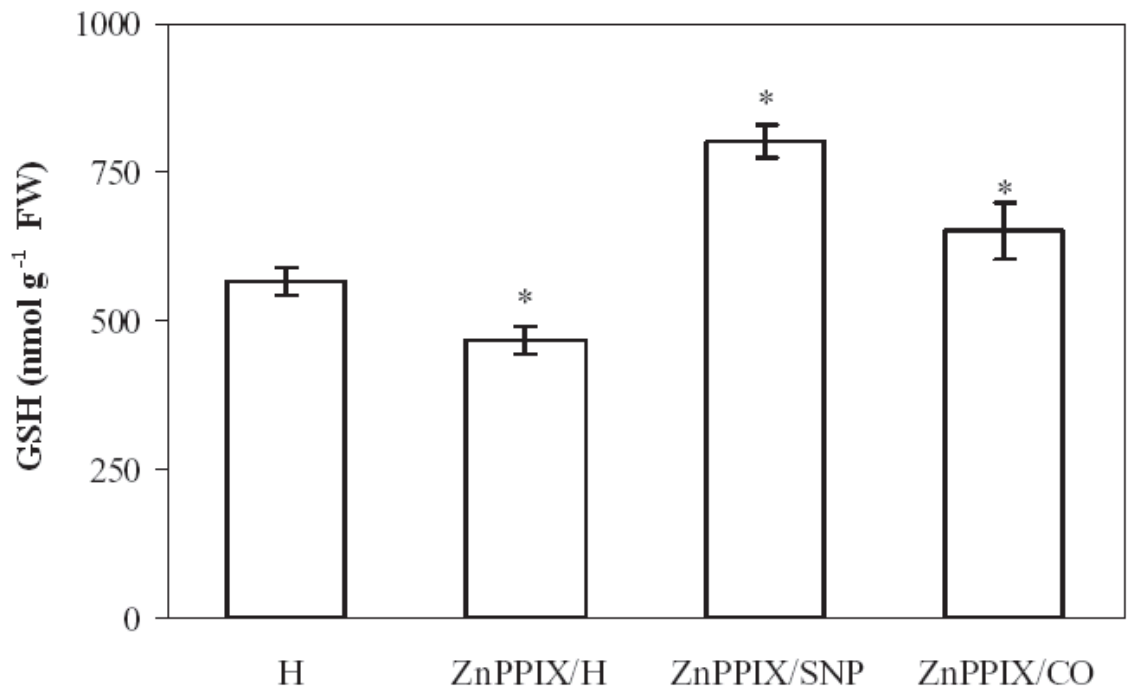


Fig. 6. Effect of NO and CO on GSH content. (H) Control plants, (ZnPPIX/H) plants pretreated with ZnPPIX and then with H; (ZnPPIX/SNP) plants pretreated with ZnPPIX and then with SNP; (ZnPPIX/CO) plants pretreated with ZnPPIX and then with CO as described in Materias and Methods. * Significant difference ($p<0.05$) according to Tukey's test.

In plants pretreated with ZnPPIX for 72 h before Hoagland (H) treatment (ZnPPIX/H), GSH level diminished 20% respect to controls (H). Figure 6 shows that NO (ZnPPIX/SNP) as well as CO (ZnPPIX/H) enhanced GSH levels (40% and 15%, respectively).

3.7.2 Glutathione reductase activity

Taking into account the fact that GSH synthesis is affected by HO-1 inhibition and NO pretreatment GR activity was determinated under the same conditions. Figure 7 indicates a

positive relationship between GSH levels and GR activity. Enzyme activity (GR) diminished 22% respect to controls when HO was inhibited, but an increase was detected in plants treated with NO and CO (33% and 26%, respectively).

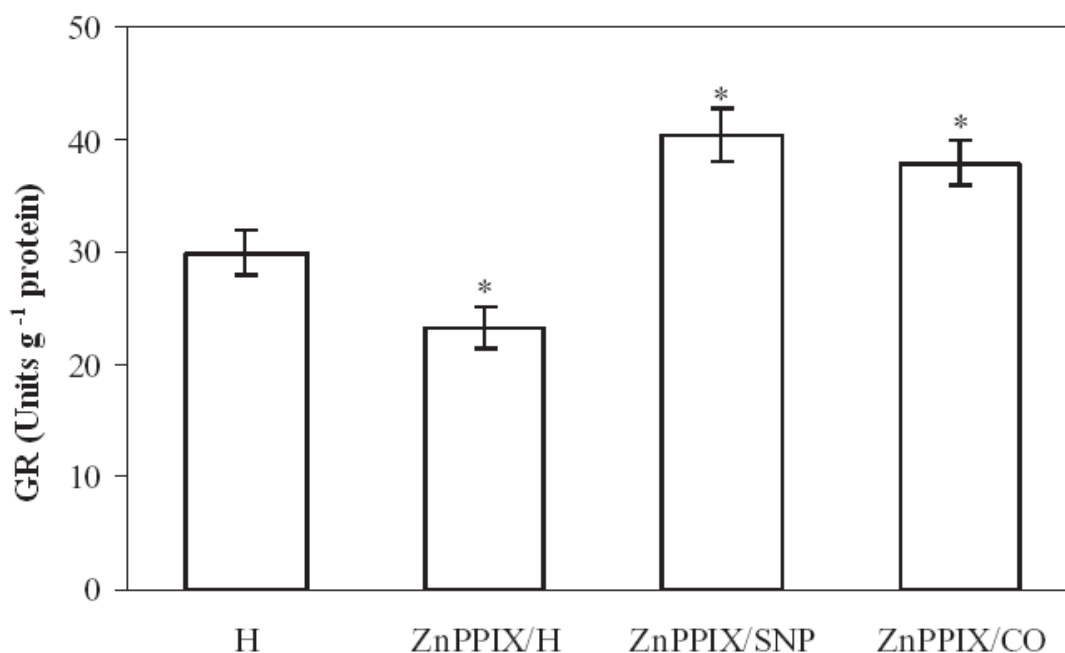


Fig. 7. Effect of NO or CO on GR activity. (H) Control plants, (ZnPPiX/H) plants pretreated with ZnPPiX and then with H; (ZnPPiX/SNP) plants pretreated with ZnPPiX and then with SNP; (ZnPPiX/CO) plants pretreated with ZnPPiX and then with CO as described in Materials and Methods. * Significant difference ($p < 0.05$) according to Tukey's test.

3.7.3 HO-1 gene expression

Figure 8 shows HO-1 gene expression under different conditions. ZnPPiX/SNP treatment brought about a 20% augmentation respect to controls. This increase is positively correlated with GSH content and GR activity. On the other hand, CO did not show any effect. It is interesting to note that the enhancement of GSH content is not related to oxidative stress, since TBARS levels in roots of SNP and CO treated plants do not differ from controls. In contrast, HO inhibition brought about an enhancement (28%) in TBARS levels.

4. Discussion

In a previous work, we found that SNP pre-treatment ameliorates Cd-induced oxidative stress and modulates HO-1 gene expression in soybean plants (Noriega et al. 2007). Taking into account the fact that NO is involved in various signaling pathways, in the present study we evaluated whether this molecule could enhance HO activity conferring a major protection against salt stress.

Our data demonstrated that, depending on its concentration, NO can improve the plant antioxidant response against salinity. This model was appropriate to determine the beneficial effect of exogenously added NO. While the lower dose of SNP did not reduce the oxidative damage (data not shown), the application of 500 or 750 μ M SNP showed a deleterious effect suggesting a pro-oxidant behavior of NO at these concentrations (Figure 1).

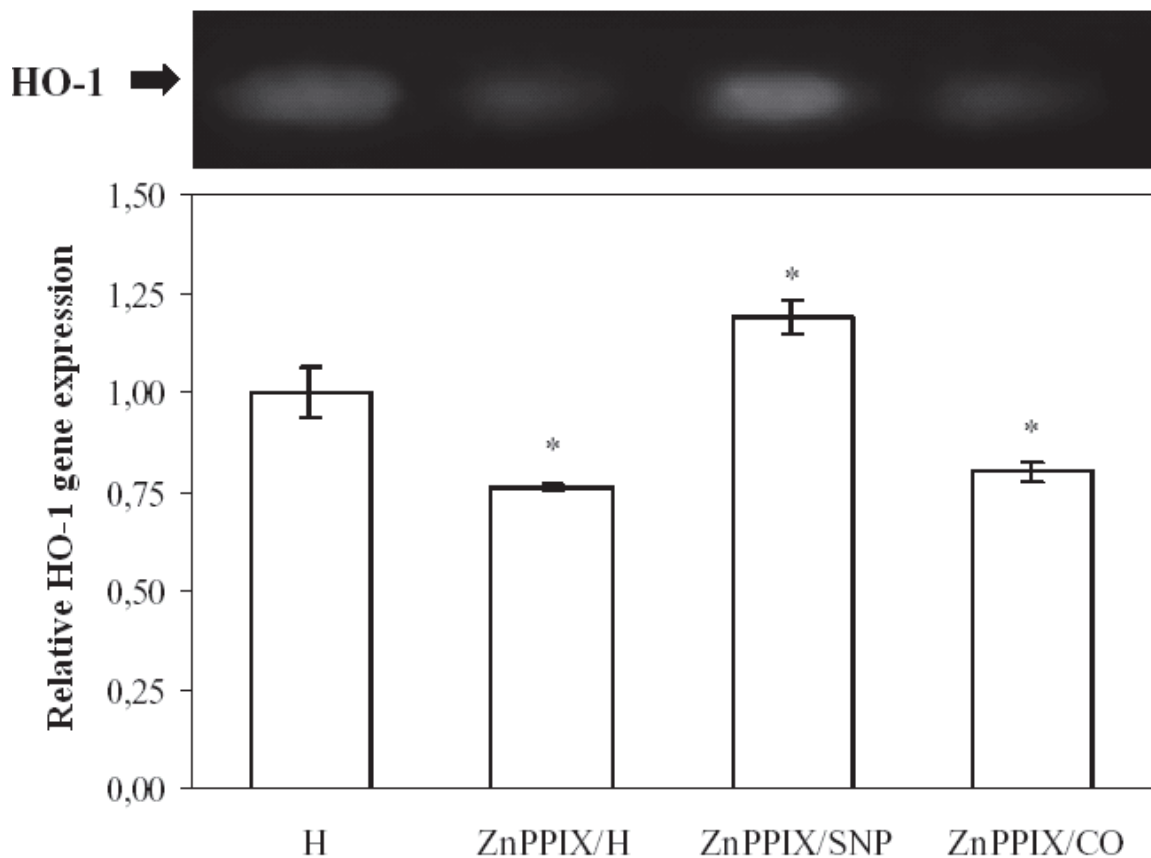


Fig. 8. Effect of NO or CO on HO-1 gene expression. (H) Control plants, (ZnPPiX/H) plants pretreated with ZnPPiX and then with H; (ZnPPiX/SNP) plants pretreated with ZnPPiX and then with SNP; (ZnPPiX/CO) plants pretreated with ZnPPiX and then with CO as described in Materials and Methods. * Significant difference ($p < 0.05$) according to Tukey's test.

The pre-treatments with 250 μ M SNP effectively ameliorated NaCl-induced oxidative stress, as indicated by the decrease in H_2O_2 and $O_2^{\cdot -}$ formation (Figure 4), preventing TBARS formation (Figure 2) and enhancing GSH content (Figure 3). The activities of classical antioxidant enzymes, such as CAT and APX were also augmented by SNP treatment, instead of the drastically diminution observed with salinity alone (Table 1). These data are in agreement with reports showing a protective effect of NO in plants subjected to other stresses (Zhao et al. 2004; Shi et al. 2005 and Noriega et al. 2007). Nevertheless, the molecular mechanism that mediates NO enhancement of antioxidant enzyme activities is not completely understood. Interestingly, we found that HO and CAT activities had a similar behaviour with respect to SNP pre-treatment under salinity (Table 1). A recent study showed that the time-course of induction of those enzymes in soybean nodules subjected to Cd stress is related (Balestrasse et al. 2008). These results suggest a close relationship between the signal transduction pathways involved in the response of HO and CAT after oxidative stress generation and support the antioxidant role of HO. In addition, there was a positive correlation between HO-1 transcript levels and enzyme activity (Figure 5 and Table 1). Previous reports have also demonstrated that the enhancement of HO activity is associated with an increase in HO-1 transcript levels and protein content (Yannarelli et al. 2006 and Balestrasse et al. 2008). Although this mechanism can account for the changes observed in HO activity, the incidence of post-translational modifications or different HO

isoforms under stress conditions needs to be addressed. Experiments carried out in plants treated with SNP in the absence of NaCl showed that NO itself can up-regulate HO-1 mRNA expression, but to a lesser extent (Figure 5). This observation indicates that a certain balance between NO and ROS is required to trigger the full response. Interestingly, a recent report found that the ROS-NO ratio is important to elicit ROS-activated stress responses and cell death regulation in plant leaves during ozone exposure (Ahlfors et al. 2009). Moreover, new evidence suggests that plastids and peroxisomes are important regulators of NO levels in plants (Corpas et al. 2009 and Gas et al. 2009).

Biliverdin, one of the products of the HO, is an efficient scavenger of ROS and it can account for the antioxidant properties of this enzyme both in animals and plants (Otterbein et al. 2003 and Noriega et al. 2004). More recently, it has been shown that CO released by HO is an important signal molecule for the tolerance mechanisms against cadmium and salt stress (Han et al. 2008). It would be interesting to determine whether CO could also play a role in the defense against salinity in soybean plants.

Pretreatment with ZnPPiX decreased HO-1 expression (Figure 8) and increased parameters of oxidative stress. When the inhibitor was added before NO or CO treatment, HO-1 expression as well as GSH content (Figure 6) and GR activity were increased (Figure 7). These results let us suppose that a close relationship between HO-1 induction and GSH content could exist. Taking together, these data provide evidence of one of the possible roles that NO, as well as CO could play against oxidative insult.

5. Conclusion

The present study together with previous results (Balestrasse et al. 2008 and Zilli et al. 2008) support the protective role of HO in soybean plants against salinity. Data here reported let us understand the mechanisms involved in HO response in NaCl-treated soybean plants. This model proposes that NO is implicated in the HO signaling pathway and, together with ROS, modulates the activity of this enzyme under salinity. In plants treated with ZnPPiX, CO did not induce HO-1, but an augmentation of GSH levels as well as GR activity was observed. On the other hand, NO not only caused a more important enhancement in GSH content and GR activity, but also brought about the induction of HO-1. Moreover, NO can enhance the antioxidant system allowing an improved plant defense to the subsequent oxidative insult. Interestingly, while NO may directly potentiate NaCl-induced HO-1 transcription, pre-treatment with SNP followed by salinity stress may protect and enhance by inducing free radical scavenging enzymes and GSH. An appropriate balance of ROS-NO is necessary to trigger the full HO response. In contrast to other stress conditions, induction of HO-1 occurs together with an enhancement of GSH levels and GR activity. In conclusion, the present study provides new insights into the molecular response of soybean plants to salinity and also evidences that HO plays an important role during stress conditions.

6. Acknowledgments

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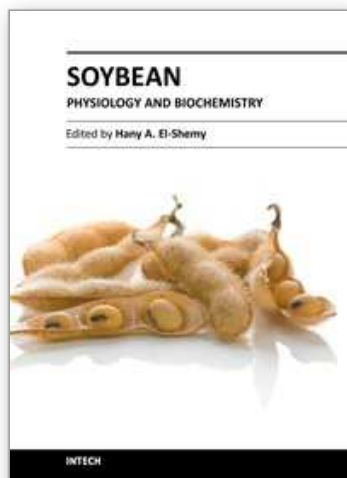
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Worldwide, soybean seed proteins represent a major source of amino acids for human and animal nutrition. Soybean seeds are an important and economical source of protein in the diet of many developed and developing countries. Soy is a complete protein and soyfoods are rich in vitamins and minerals. Soybean protein provides all the essential amino acids in the amounts needed for human health. Recent research suggests that soy may also lower risk of prostate, colon and breast cancers as well as osteoporosis and other bone health problems and alleviate hot flashes associated with menopause. This volume is expected to be useful for student, researchers and public who are interested in soybean.

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