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EFFECT OF SODIUM NITROPRUSSIDE ON THE GERMINATION AND ANTIOXIDANT ACTIVITIES OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL)

S. HAYAT[1,2*], S. YADAV[2], M. N. ALYEMENI[1] and A. AHMAD[2]
[1]*King Saud University, Department of Botany & Microbiology, Riyadh, Saudi Arabia*
[2] *Aligarh Muslim University, Plant Physiology Section, Department of Botany, Aligarh 202 002, India*

Abstract

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Seeds of tomato were soaked in double distilled water (control) or sodium nitroprusside (SNP; 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} or 1.0 M) a nitric oxide (NO) donor, for 8h. SNP significantly affected the seed germination, NR activity and seedling growth in a concentration dependent manner. The concentrations ranging from 10^{-8} to 10^{-5} M favored these parameters, whereas, the higher concentration of SNP (1M) showed an inhibitory effect. However, proline content and antioxidant enzyme exhibited an increase in response to 1M of SNP. It is concluded from the present investigation that nitric oxide acts as a stimulator at low concentrations whereas at higher concentration it acts as a stress inducer.

Key words: growth, germination, nitrate reductase activity, proline, tomato

Introduction

Nitric oxide (NO) is an atmospheric gaseous molecule soluble in water and lipids. Research on NO, in plants has gained a considerable attention in recent years and there is increasing evidence of a role of this molecule in plants. Besides its presence in atmosphere, NO is also formed endogenously mainly in young actively growing tissues. NO production in plant tissues was first observed by Klepper (1975) and later on four different enzymatic pathways involved in its production have been proposed; (a) nitric oxide synthase, (b) plasma membrane bound nitrate reductase, (c) mitochondrial electron transport chain and (d) non-enzymatic reactions (Guo et al., 2003). Due to its highly lipophilic nature, NO acts as an intra and intercellular signaling plant growth regulator (Beligni and Lamattina, 2000) that mainly acts against the oxidative stress (Neill et al., 2002). NO at low concentrations has been found to be involved in regulation of diverse biochemical and physiological processes in plants (Kopyra and Gwozdz, 2003; Neill et al., 2003). However, at higher concentrations, NO may prove to be potentially toxic to the plant systems (Beligni and Lamattina, 2000). Keeping these points in view, the present experiment was designed with an objective to evaluate the effect of a range of concentrations of SNP, a nitric oxide donor on the performance of tomato.

Materials and Methods

Healthy seeds of tomato (*Lycopersicon esculentum* Mill var K-21) were surface sterilized with 0.01% mercuric chloride followed by repeated washings with double distilled water (DDW) to remove the adhered mercuric chloride particles on the seed surface. These seeds were then soaked for 8 hours either in DDW (control) or sodium nitroprusside (SNP; 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, or 1.0 M), a nitric oxide donor at 27 ± 2^{o}C under dark conditions. Afterwards, seeds were transferred to sterile Petri-plates and allowed to germinate in a growth chamber at 27 ± 2^{o}C. The seed germination was recorded at 96 h. The resulting seedlings were sampled on day 7 to assess the following parameters.

Length of root and shoot

The length of the root and shoot from each treatment was measured on a meter scale.

Nitrate Reductase Activity

Nitrate reductase (NR) activity was measured by adopting the method of Jaworski (1971). The fresh leaf samples were cut into small pieces and transferred to plastic vials containing phosphate buffer (pH 7.5) followed by the addition of potassium nitrate and isopropanol solutions. The reaction mixture was incubated at 30℃, for 2h followed with the addi-

*Corresponding author, e-mail: hayat_68@yahoo.co.in

tion of N-1-naphthyletylenediamine dihydrochloride and sulphanilamide. The absorbance was read at 540 nm.

Leaf Proline Content

The proline content in fresh leaf was determined by the method given by Bates et al. (1973). The samples were extracted in sulphosalicylic acid. To the extract, an equal volume (2 mL) of glacial acetic acid and ninhydrin solutions were added. The sample was heated at 100°C, to which 5 mL of toluene was added after cooling in ice bath. The absorbance by toluene layer was read at 528 nm, on a spectrophotometer (Spectronic-20D, Milton Roy, USA).

Antioxidant Enzymes

Fresh leaves (0.5 g) were homogenized with 5 mL of 50 mM phosphate buffer (pH 7.0) containing 1% PVP (polyvinylpyrrolidone). The homogenate was centrifuged at 10000 × g for 10 min. The supernatant was collected and used as a source for enzyme assay. This whole extraction process was carried out at 4°C. The assay of peroxidase (POX) and catalase (CAT) was done by adapting the method of Chance and Maehly (1956). Activity of CAT was measured by titrating the reaction mixture [phosphate buffer (pH 6.8), 0.1 M H_2O_2, enzyme extract and 2% H_2SO_4] against 0.1 N $KMnO_4$. The activity of POX was measured by observing the change in the absorbance of reaction mixture [pyrogallol phosphate buffer (pH 6.8), 1% H_2O_2 and enzyme extract], due to catalytic conversion of pyrogallol to perpurogallin at an interval of 20 s for 2 min at 420 nm. A control set was prepared by using DDW instead of enzyme extract.

The activity of superoxide dismutase (SOD) was assayed by measuring its ability to inhibit the photochemical reduction of nitrobluetetrazolium (NBT) by using the method of Beauchamp and Fridovich (1971). The reaction mixture [50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 0.1 mM EDTA and 0-50 µl enzyme extract] in tubes were placed under 15 W fluorescent lamps for starting the reaction. After 10 min, the reaction was stopped by switching off the light. Non-illuminated reaction mixture was used as a blank. The absorbance was measured at 560 nm and the SOD activity was expressed as unit g^{-1} fresh mass. One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of NBT photo-reduction.

Statistical analysis

The experiment was conducted according to simple randomized block design. A total of ten replicates for each treatment were taken. Treatment means were compared by analysis of variance using SPSS (SPSS, Chicago, IL, USA). Least Significance Difference (LSD) was calculated at the 5% level of probability. Standard error due to replicate was calculated.

Results

Seed germination and length of root and shoot

The treatments of SNP as seed soaking significantly affected the germination, length of root and shoot as compare to water soaked control (Table 1). Lower concentrations of SNP (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, or 10^{-8} M) enhanced the germination percent, length of root and shoot significantly over the control, whereas, high concentrations (10^{-3}, 10^{-2}, 10^{-1}, or 1.0 M) inhibit the seed germination and length of root and shoot and maximum inhibition was recoded when seeds were soaked in 1 M of SNP. Out of the various concentrations used, 10^{-5} M SNP showed best results and increased the germination percent by 31.8%, root length 35.0% and shoot length 42.0% when compared to water soaked control.

Nitrate reductase (NR) activity

The activity of NR decreased in the seedlings raised from the seeds soaked in high concentrations (10^{-4}, 10^{-3}, 10^{-2}, 10^{-1},

Table 1
Effect of pre-sowing seed soaking of sodium nitroprusside (SNP) on germination (%) and length of root and shoot of tomato. Data are the mean of five independent replicates ± standard error

SNP Concentration (M)	Germination, %	Root length, cm	Shoot length, cm
Control (water soaked)	73.30±4.65	3.58±0.19	2.99±0.41
1.0 M	66.65±3.06	2.13±0.06	2.56±0.25
10^{-1} M	66.65±3.39	2.99±0.37	2.79±0.17
10^{-2} M	66.90±2.94	3.35±0.18	2.96±0.23
10^{-3} M	68.30±3.27	3.50±0.15	3.04±0.38
10^{-4} M	86.65±4.72	3.66±0.12	3.94±0.36
10^{-5} M	96.60±3.99	4.81±0.08	4.23±0.18
10^{-6} M	82.12±2.56	3.96±0.19	3.27±0.18
10^{-7} M	80.00±3.71	3.82±0.08	3.27±0.25
10^{-8} M	78.30±2.70	3.71±0.12	3.10±0.18

or 1.0 M). However, the low concentrations (10^{-5},10^{-6}, 10^{-7}, or 10^{-8} M) of SNP increased the NR activity, out of which 10^{-5} M SNP caused maximum increase of 24.0% compared to the water soaked control (Table 2).

Proline content

The seedlings raised from the seeds soaked in various concentrations (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} 10^{-3}, 10^{-2}, 10^{-1}, or 1.0 M) of SNP showed increase in the accumulation of proline compared to the water soaked control (Table 2). The highest concentration (1.0 M) of SNP showed maximum proline accumulation of 80.6% in the seedlings compared to water soaked control.

Antioxidant enzymes

The high concentration (10^{-3}, 10^{-2}, 10^{-1}, or 1.0 M) decreased the activity of peroxidase (POX) and catalase (CAT) enzymes while low concentration (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, or 10^{-8} M) enhanced the activity of these enzymes (Table 3). Out of the various concentrations, 10^{-5} M SNP proved best and increased the activity of POX by 31.5% and CAT 30.9% compared to water soaked control. The activity of superoxide dismutase (SOD) enzyme increased in the seedlings raised from the seed soaked in different concentrations (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} 10^{-3}, 10^{-2}, 10^{-1}, or 1.0 M) of SNP with respect to water soaked control (Table 3). Out of these concentrations, 10^{-5} M SNP caused maximum increase of 108.6% in the SOD activity compared to the control.

Discussion

Nitric oxide, a bioactive signaling molecule, is known to play a pivotal role in a number of plant physiological processes, such as regulating growth and development, induction of seed germination etc. (Hayat et al., 2009). In the present study, SNP, an NO donor at lower concentration (10^{-5} M) proved fruitful and enhanced the seed germination percent, growth, activities of NR and antioxidant enzymes and pro-

Table 2
Effect of pre-sowing seed soaking of sodium nitroprusside (SNP) on NR activity [n mole NO_2 kg^{-1}(F.M.) h^{-1}] and proline content [mg g^{-1} (F.M.)] of tomato. Data are the mean of five independent replicates ± standard error

SNP Concentration (M)	NR activity	Proline content
Control (water soaked)	156.95±11.08	5.27±0.52
1.0 M	86.32±9.76	9.52±0.80
10^{-1} M	94.17±8.34	7.70±0.61
10^{-2} M	109.86±9.30	7.20±0.65
10^{-3} M	125.56±11.49	7.01±0.52
10^{-4} M	145.18±13.26	6.99±0.41
10^{-5} M	206.25±18.43	6.08±0.60
10^{-6} M	168.36±12.9	6.27±0.51
10^{-7} M	163.13±13.4	6.88±0.59
10^{-8} M	158.17±12.1	6.92±0.81

Table 3
Effect of pre-sowing seed soaking of sodium nitroprusside (SNP) on POX [g^{-1}(F.M.)], CAT [µmol H_2O_2 decomposed g^{-1} (F.M.)] and SOD (units g^{-1} FM) activities of tomato. Data are the mean of five independent replicates ± standard error

SNP Concentration (M)	POX	CAT	SOD
Control (water soaked)	7.30±0.65	178±21.23	35±2.89
1.0 M	6.65±0.56	145±15.65	55±4.76
10^{-1} M	6.55±0.49	162±17.25	59±6.23
10^{-2} M	6.20±0.51	165±18.56	54±6.12
10^{-3} M	6.80±0.54	169±17.45	56±6.66
10^{-4} M	8.65±0.68	224±21.25	66±7.12
10^{-5} M	9.60±0.78	233±24.45	73±5.85
10^{-6} M	8.22±0.79	216±18.93	67±6.92
10^{-7} M	8.00±0.83	193±21.25	65±5.65
10^{-8} M	7.83±0.68	176±16.85	62±6.45

line content in tomato seedlings (Tables 1-3). Different NO donors have been known to stimulate seed germination in tree (Giba et al., 1998), lettuce (Beligni and Lamattina, 2000) and *Arabidopsis* (Libourel et al., 2006). It has been reported that the seed aleurone layer is the site for NO perception, synthesis and NO mediated responses. NO releases the ABA controlled dormancy by increasing the activity of ABA degrading enzymes (Bethke et al., 2006) with the initiation of GA-stimulated germination (Bethke et al., 2007). The growth (root and shoot length) of plants resulting from seeds pretreated with lower concentrations of SNP (10^{-8}-10^{-5} M) was higher than that of the control (Table 1). The possible reason is that NO favors the exo- and endo-β-D-glucanase activity in the cell wall (Terasaki et al., 2001). It has been further verified by using NO-deficient mutants, where enzyme activity decreased and growth remained restricted. The glycosidic linkage between glucose units within the cell wall is broken by these enzymes to favor loosening and to an increase its extensibility (Zhang et al., 2003). This drives growth by increasing internal turgor pressure, which could be generated by an increase in relative water content (Hayat et al., 2013). However, 1 M SNP inhibited plant growth that could have been a consequence of a supra-optimal concentration of SNP. Similar observations have been reported earlier (Beligni and Lamattina, 2000).

An increase in NO concentration has a synergistic effect with that of the reactive oxygen species (Beligni and Lamattina, 1999) that causes damage to the cell at multiple points, including the membrane, by generating oxidative stress. The advent of oxidative stress in the present investigation is evident from an increase in proline content by 1M SNP (Table 2). Oxidative stress is recognized to damage the plasma membrane and consequently allows the influx and efflux of nutrients such as NO_3, which is an inducer of nitrate reductase (Campbell, 1999). Membrane damage could result in a decrease in NO_3 concentration. Therefore, the level of NR in plants pre-treated with 1 M SNP was lower than that of the control (Table 2). However, SNP at lower concentration (10^{-5} M) acted as an antioxidant and thus played a protective role (Beligni and Lamattina, 1999). The reason behind this is that NO stimulates the post translational regulatory pathways of NR (Jin et al.,2009) that could also be markedly enhanced by NAA (an analogue of IAA) in chicory (Vuylsteker et al., 1998), cytokinin benzyladenine in *Arabidopsis* (Yu et al., 2001), and by salicylic acid in maize (Jain and Srivastava, 1981). The mechanism of action of these chemical compounds in regulating NR is however poorly understood. Moreover, all the effects of chemicals lead to generation of NO (Pagnussat et al., 2004). Increased activity of NR by foliar application of NO is also reported earlier (Hayat et al., 2011).

The seed soaking treatment of SNP (10^{-5} M) enhanced the activities of antioxidant enzymes (CAT, POX, SOD) in the tomato seedlings (Table 3). Similar results have been reported in canola leaves (Kazemi et al., 2010) and wheat roots (Wang et al., 2010) by the application of NO. The enhanced activity of these enzymes by NO may be due to NO-mediated increased availability of iron in plants and improved biosynthesis of these enzymes (Kazemi, 2012). Moreover, it has been reported that NO acts as an antioxidant and quenches ROS that is generated during oxidative stress (Xiong et al., 2010). This suggests that treatment with NO on tomato plants could directly or indirectly activate antioxidant enzymes. Different metal-induced proline syntheses are associated with NO generation (Zhang et al., 2008). The application of SNP increased activity and transcripts level of delta 1-pyrroline-5-carboxylate synthase (P5CS), a key enzyme of proline synthesis, by increasing the intracellular level of NO. Moreover, the rise in proline in the present study (Table 2) is proposed to be generated through either a slowdown in its hydrolysis or the diversion of protein synthesis toward accumulation of proline (Irigoyen et al., 1992).

Conclusions

In brief, the results in the present study indicate that SNP shows dual behavior. It acts as a stimulator at low concentrations whereas at higher concentration it acts as a stress inducer, which was reflected from the increased levels of proline in response to the highest concentration (1M) of nitric oxide.

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