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Research Article

Effect of Treatment with Different Concentrations of Sodium Nitroprusside on Survival, Germination, Growth, Photosynthetic Pigments and Endogenous Nitric Oxide Content of *Lupines termis* L. Plants

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Abstract

The current study was conducted to investigate the impact of exogenous application of different concentrations of sodium nitroprusside (0, 0.2, 0.4, 0.6 and 0.8 mM) as NO donor on germination, survival, growth, photosynthetic pigments and the level of endogenous NO of *Lupinus termis* L. plants. The results proved that seeds treatment with sodium nitroprusside negatively affected percentage of seed germination and plant survival. On the other hand, the effect of foliar spray of sodium nitroprusside on lupine plants was concentration-dependent. Lower concentrations (0.2 up to 0.6 mM) had a promotive effect on all the measured growth parameters and photosynthetic pigments content whereas higher concentration (0.8 mM) was inhibitory. All the applied concentrations of sodium nitroprusside increased the level of endogenous NO indicating that NO released in plant cells by SNP treatments.

Keywords: Growth; Lupine; Nitric Oxide; Photosynthetic Pigments; Sodium Nitroprusside

Introduction

Nitric oxide (common name) or nitrogen monoxide (systematic name) is one of the smallest diatomic molecules with a chemical formula NO. Displaying hydrophobic properties, NO may easily migrate in the hydrophilic regions of the cell, such as the cytoplasm, it can also freely diffuse through the lipid phase of membranes [1].

NO was clearly shown, as an important signal molecule involved in plant response to different environmental stresses, by the late 1990's [2,3]. All these findings started an era of new and challenging studies [4]. Many possible sources of NO were detected; the physiological role of each source depends on the species, type of tissue or cells, external conditions and potential activation of the signal pathway in the plant [5]. NO can be formed both enzymatically and non-enzymatically, in biological systems. Nitric oxide can be produced by four routes in plants: (i) nitric oxide synthase, (ii) plasma membrane-bound nitrate reductase, (iii) mitochondrial electron transport chain, or (iv) non-enzymatic reactions. The major origin of NO production in plants is probably through the action of NAD(P)H-dependent nitrate or nitrite (NiR) reductases [6].

NO is an important signaling molecule with different physiological functions in plants. NO plays a vital role in diverse physiological functions in plants, such as seed dormancy and photosynthesis [7].

The current investigation was carried out to determine the effect of treatment with different concentrations of sodium nitroprusside (as a NO donor) on survival, germination, growth and photosynthetic pigments content of *Lupines termis* L. plants. Moreover, the impact of exogenous NO application on endogenous nitric oxide content in shoots and roots of lupine plants were also detected. The most effective concentration of sodium nitroprusside that has positive stimulatory effect on lupine plants was determined

Materials and Methods

Plant materials and growth conditions

Lupinus termis L. seeds were obtained from the Agriculture Research Center, Ministry of Agriculture, Giza, Egypt.

Uniform seeds of *Lupinus termis* L. were surface sterilized with 2.5% sodium hypochlorite for 15 minutes and washed thoroughly with distilled water. The seeds were planted in plastic pots (10 seeds per pot; 22 cm diameter and 20 cm long) filled with 4 kg mixture of soil (2:1, clay to sand, w/w). The pots were divided into 2 groups. Pots of the first group were irrigated with tap water to serve as a control; the second group was divided into 4 subgroups, each irrigated with one of the following concentrations of sodium nitroprusside (SNP; 0.2, 0.4, 0.6 and 0.8 mM) as a NO donor. After one month the percentage of germinated seeds and survived plants were calculated.

To study the effect of NO on growth, photosynthetic pigments and endogenous NO contents in lupine plants, 3-weeks old lupine seedlings were foliar sprayed with different concentrations of SNP (0, 0.2, 0.4, 0.6 and 0.8 mM). The pots were kept in green house, where the plants subjected to natural day/night conditions during the experimental period with normal photoperiod and average 28°C day and 16°C night temperature. Pots were kept at 80% water saturation capacity till the end of the experiment. Samples were collected from 2 months-old plants to measure growth criteria, photosynthetic pigments and nitric oxide contents.

Extraction and estimation of photosynthetic pigments

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined in the leaves of the investigated plant. The Spectrophotometric method recommended by Metzner., *et al.* was used [8]. A known fresh weight of leaves was homogenized in 85% aqueous acetone for 5 minutes. The homogenate was centrifuged and the supernatant was made up to volume with 85% aqueous acetone. The extinction was measured against a blank of pure 85% aqueous acetone at 3 wave lengths of 452.5,644 and 663 nm using Spectrophotometer. Taking into consideration the dilution made of the pigment fraction (chlorophyll a, chlorophyll b and carotenoids) were determined as µg ml^{-1} using the following equations:

Chlorophyll a =10.3 E_{663} - 0.918 E_{644} = µg ml^{-1}
Chlorophyll b =19.7 E_{644} - 3.8718 E_{663} = µg ml^{-1}

Carotenoids = 4.2 $E_{452.5}$ - (0.0264 Chlorophyll a + 0.4260 Chlorophyll b) = µg ml^{-1}

Finally, the pigment contents were calculated as µg g^{-1} dry weight of leaves.

Estimation of Nitric oxide content

The Griess reaction is one of the most widely used assays for NO detection and represents the basic reaction of relatively cheap commercial kits for NO measurements. The technique was pioneered by Johann Peter Griess (1829 - 1888), a German organic chemist, who was one of the founders of the azo and diazo dye industry. Griess suggested that nitrites could be detected by reacting with sulphanilic acid and α-naphthylamine under acidic conditions to yield an azo dye. Sulphaniliamide and N-naphthyl-ethylenediamine (NED) are used to react with NO_2. The resulting stable water-soluble azodye may be quantified by measuring spectrometric absorption at 520 nm. NO can be readily oxidized to NO_2 so that the basic Griess reaction is used as an indirect assay for NO. Sun., *et al.* [9] described how to determine nitric oxide content, sulfanilamide is used as a diazotizing reagent, in acidic media to form a transient diazonium salt. After incubation for 5 - 10 minutes, this intermediate is then allowed to react with a coupling reagent N-naphthyl-ethylenediamine (NED) after 10 minutes. to form a stable azo compound, 2 ml of plant extract was added to 2 ml of Griess reagent and measured at 540 nm, a calibration curve using sodium nitrite standard was made to calculate the nitric oxide content of samples.

Statistical analysis

Mean values were calculated from measurements of five replicates and standard deviations of the means were analyzed using independent samples T- test (SPSS program 17.0), to test for significant difference between means, two tailed P-value [10].

Results and Discussion

Percentage of germination and survival

It is apparent from figure 1 that the percentage of germination of *Lupinus termis* L. was the least in case of applying SNP 0.8 mM (30%), while the highest percentage was detected in case treatment with SNP 0.4 mM (80%), followed 0.6 mM (75%) then 0.2 mM (60%) as compared with the percentage of the respective control.

The percentage of survived plants reached the maximum value in case of treatment with SNP 0.4 and 0.6 mM by 65% and 55% respectively (Figure 1) after 30 days of sowing compared to the control value, while complete death occurred when lupine plants were treated with 0.8 mM SNP. Matching our results, exogenously applied NO was proved to delay the process of germination of rice seedlings at a concentration of 1 mM [11]. Bethke., *et al.* [12] suggested that NO could decrease ABA and GA ratio in seeds necessary to release inhibition of seed germination induced by salinity or break the dormancy. NO is considered as a likely player of a signaling pathway that promotes loss of dormancy and has been suggested to behave as an endogenous regulator of this process [13].

Figure 1: Effect of different concentrations of sodium nitroprusside (SNP) on percentage of germination and survival of *Lupinus termis* L.

Growth characteristics

The change in the growth parameters of shoots and roots of *Lupinus termis* L. in response to treatment with different concentrations of SNP (0.0, 0.2, 0.4, 0.6 and 0.8 mM) as a NO donor, are given in table 1.

Growth characteristics / Treatment		Shoot length (cm)	Root length (cm)	No. of leaves/plant	Area of leaves (cm^2)	Fresh wt. of shoot (g)	Dry wt. of shoot (g)	Fresh wt. of root (g)	Dry wt. of root (g)
Control (H_2O)		11.50 ± 0.00	7.27 ± 0.14	12.33 ± 0.33	64.73 ± 4.30	4.23 ± 0.12	0.52 ± 0.02	1.96 ± 0.04	0.31 ± 0.00
SNP	0.2 mM	12.21 ± 0.14*	8.43 ± 0.06*	12.67 ± 0.33	60.63 ± 4.44	4.49 ± 0.22	0.71 ± 0.03*	2.23 ± 0.43	0.32 ± 0.01
	P value	0.04	0.007	0.519	0.543	0.370	0.015	0.595	0.581
	0.4 mM	14.53 ± 0.03*	11.33 ± 0.44*	13.00 ± 0.00	71.89 ± 3.93	5.05 ± 0.44	0.99 ± 0.08*	3.19 ± 0.09*	0.42 ± 0.01*
	P value	0.00	0.007	0.184	0.287	0.199	0.025	0.001	0.009
	0.6 mM	14.17 ± 0.17*	10.00 ± 0.50*	12.33 ± 0.33	68.57 ± 4.22	4.15 ± 0.27	0.76 ± 0.04*	3.07 ± 0.14*	0.34 ± 0.01
	P value	0.004	0.024	1.00	0.559	0.812	0.012	0.009	0.147
	0.8mM	10.57 ± 0.03*	8.50 ± 0.50	11.33 ± 0.33	49.12 ± 0.10	3.05 ± 0.17*	0.46 ± 0.03	1.60 ± 0.07*	0.32 ± 0.02
	P value	0.001	0.123	0.101	0.068	0.007	0.177	0.019	0.893

Table 1: Effect of different concentrations of sodium nitroprusside on growth characteristics of *Lupinus termis* L. Data are mean ± SE (Standard Error) of five replicates.
Values with a superscript (*) are significantly different from the control at P < 0.05 (significant).

Shoot length: It is apparent from the obtained data that, shoots length of *Lupinus termis* L. increased by increasing the applied concentrations of SNP (0.2, 0.4 and 0.6 mM), the maximum length was obtained in case of the treatment with 0.4 and 0.6 mM where it showed significant increases by (26.35%) and (23.22%) respectively over the control value, the shortest shoot length was observed response to 0.8 mM (8.09% below the control value).

Root length: All the applied concentrations of SNP increased root length of lupine plant as compared with the corresponding control.

Number of leaves per plant: The maximum number of leaves was obtained by using 0.4 mM SNP (5.43% over the control value). Meanwhile, sodium nitroprusside 0.8 mM showed 8.11% decrease below the control value.

Area of leaves: Only 0.4 and 0.6 mM SNP caused an increase in the area of leaves of *Lupinus termis* L. as compared with the corresponding control, reaching the highest value (11.06%) over the control value, in case of 0.4 mM sodium nitroprusside.

Fresh weight of shoot: 0.2 and 0.4 mM SNP have a stimulatory effect on fresh weight of shoot of lupine, showing the highest increase in response to treatment with 0.4 mM by (19.38%) compared with the control value. Whereas, 0.6 mM and 0.8 mM SNP showing a decrease in the fresh weight of shoot by (1.89%) and (27.89%) respectively below the control value.

Dry weight of shoot: The most stimulatory effect in dry weight of shoots was detected in response to 0.4 mM and evaluated by 90.38% over the corresponding control value, the only decrease in the dry weight of shoot in SNP-treated plants was noticed in plant subjected to 0.8 mM and was calculated by 11.54% below the control value.

Fresh weight of root: The fresh weight of roots of lupine plants increased progressively in most of the SNP treatments as compared with the corresponding control value, showing the highest significant increase when applying 0.4 mM, the increase were evaluated by 62.76% over the control value.

Dry weight of root: as compared with control value, all the applied concentrations of SNP increased dry weight of roots estimated by 35.48% and 9.68% in response to 0.4 mM and 0.6 mM, respectively over the control value.

Generally, the effect of NO on lupine growth is concentration dependent, 0.4 mM and 0.6 mM SNP caused significant increase whereas 0.8 mM SNP caused significant decrease in almost all the measured growth parameters when compared to water as the control value. A similar dual behavior of NO donor SNP was also noted by several authors, treating wheat seedlings with lower concentration of SNP promoted root growth whereas higher concentration was inhibitory [14]. Seedlings of canola, raised from the seeds treated with lower concentration of SNP, had more root length and dry mass whereas higher concentration reduced the values of these parameters [15].

Photosynthetic pigments

All the treatments of SNP caused a consistent increase in chlorophyll (a), except for 0.8 mM sodium nitroprusside that showed a decrease by 40.78% when compared to corresponding control value (Table 2). The highest increase (18.73%) was pronounced in case of treatment with 0.4mM SNP. Chlorophyll b content also showed a progressive increase when applying SNP, the most significant increase in chlorophyll b, was recognized in response to 0.4 mM and was evaluated by 214.13% as compared with the control value. Regarding carotenoids a great increase was detected when

applying all of the treatments, showing a significant increase in case of treatment with 0.4 mM sodium nitroprusside by 101.07% as compared with the corresponding control value. Consequently, total photosynthetic pigments content showed an increase response to 0.2 to 0.6 mM SNP, the highest increase (73.39%) was noticed in case of applying 0.4 mM followed by 0.6 mM with 58.46% increase over the control value.

Photosynthetic pigments / Treatment		Chlorophyll a	Chlorophyll b	Carotenoids	Total photosynthetic pigments
Control (H_2O)		3.31 ± 0.46	0.92 ± 0.08	1.86 ± 0.07	6.09 ± 0.56
SNP	0.2 mM	3.45 ± 0.24	1.02 ± 0.27	2.25 ± 0.09*	6.73 ± 0.59
	P value	0.795	0.749	0.034	0.471
	0.4 mM	3.93 ± 0.17	2.89 ± 0.47*	3.74 ± 0.23*	10.56 ± 0.58*
	P value	0.309	0.050	0.009	0.005
	0.6 mM	3.83 ± 0.06	2.47 ± 0.23*	3.35 ± 0.14*	9.65 ± 0.34*
	P value	0.370	0.014	0.003	0.009
	0.8 mM	1.96 ± 0.49	2.12 ± 0.15*	2.09 ± 0.29	6.18 ± 0.79
	P value	0.117	0.006	0.496	0.929

Table 2: Effect of different concentrations of sodium nitroprusside on photosynthetic pigments content of *Lupinus termis* L. The results are expressed in µg g-1 fresh weight. Data are mean ± SE (Standard Error) of five replicates. Values with a superscript (*) are significantly different from the control at P < 0.05 (significant).

Similarly, NO has been found to enhance chlorophyll content in potato, lettuce, and *Arabidopsis*. Also, treatment with SNP delayed yellowing and retarded the chlorophyll degradation in broccoli [16], and improved the rate of photosynthesis, chlorophyll content, transpiration rate and stomatal conductance in cucumber seedlings [17]. Moreover, plants receiving NO showed higher values for net photosynthesis, stomatal conductance and intercellular CO_2 concentration, PSII activity and Rubisco activity compared to control plants in the absence or presence of salt. Application of 100 µM NO in plants increased net photosynthesis by 66.0%, stomatal conductance by 31.0%, intercellular CO_2 concentration by 38.0%, maximal PSII photochemical efficiency by 18.0% and Rubisco activity by 38.0% in comparison to control plants [18].

Endogenous NO

It is important to determine the actual effect of SNP as a NO donor on endogenous NO content of plant because the commonly cited statement that "donors are compounds that spontaneously break down to release NO" need to be verified due to the fact that the process of donor decomposition depends on the numerous external factors. It may be additionally stimulated or inhibited by live plant tissue, thus it is necessary to take into consideration these aspects and monitor the amount of NO released by the donor [19].

Data in table 3 that all the applied concentrations of SNP markedly increased endogenous level of NO in both shoots and roots of lupine plants. The highest significant increase detected when applying 0.8 mM and was estimated by 224.24%, in shoot and 517.35% in case of roots as compared to the control value. According to Grossi and D'Angelo [20] the mechanism of release of NO molecule from SNP is hypothesized to involve the sulfhydryl-containing compounds glutathione and cysteine, leading to the formation of the corresponding disulfides and S-nitrosothiols, NO, and cyanide ions. Previous studies proved that, when plants were treated with NO only, NR activity in leaves was positively modulated by NO released from SNP or $NaNO_2$ [21].

Nitric oxide content / Treatment		Shoot	Root
Control (H_2O)		185.75 ± 1.04	113.87 ± 1.01
SNP	0.2 mM	303.81 ± 17.60*	150.40 ± 2.07*
	P value	0.021	0.001
	0.4 mM	361.57 ± 2.62*	299.60 ± 24.38*
	P value	0.00	0.017
	0.6 mM	445.77 ± 3.43*	378.29 ± 10.96*
	P value	0.00	0.002
	0.8 mM	602.28 ± 11.03*	702.98 ± 39.12*
	P value	0.001	0.004

Table 3: Effect of different concentrations of sodium nitroprusside, on endogenous nitric oxide content of *Lupinus termis* L. The results are expressed in mg g-1 fresh weight. Data are mean ± SE (Standard Error) of five replicates. Values with a superscript (*) are significantly different from the control at P < 0.05 (significant).

Conclusion

The present work proved that, the effect of exogenous application of SNP (as a NO donor) on growth parameters and photosynthetic pigments of lupine plants is concentration – dependent. Lower concentrations (0.2 up to 0.6 mM) has a promotive effect whereas higher concentration (0.8 mM) was inhibitory. The maximum stimulatory effect was detected in response to treatment with 0.4 mM followed by 0.6mM SNP. We also confirmed that, fo-

liar spray with SNP increases endogenous NO content in plants in a concentration-dependent manner.

Conflict of Interest

Authors declare that they have no conflict of interest.

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