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ENHANCING GROWTH IN *EUCOMIS AUTUMNALIS* (MILL.) CHITT. SEEDLINGS WITH EXOGENOUS APPLICATION OF NITRIC OXIDE

Short communication

Piotr SALACHNA*, Agnieszka ZAWADZIŃSKA, Łukasz WIERZBIŃSKI,
Wojciech SENDEREK
West Pomeranian University of Technology in Szczecin
Papieża Pawła VI 3, 71-459 Szczecin, Poland

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ABSTRACT

Nitric oxide (NO) is a molecule that regulates biological processes in plants and may be used in horticultural practice as a potential plant growth regulator. *Eucomis autumnalis* (Mill.) Chitt., also known as pineapple lily, is a member of the Asparagaceae and native to South Africa. Pineapple lily are well suited for production as pot plants, cut flowers, and garden plants. The potential also exists for this species to be used as a medicinal plant. The aim of the study was to assess the effects of the NO donor of sodium nitroprusside (SNP) on morphological features of *E. autumnalis* seedlings. The plants grown in a greenhouse were drenched four times, at weekly intervals, with SNP solutions of the following concentration: 0, 100, 200, 300, 400, and 500 μM. The study revealed that the application of SNP significantly improved greenness index of leaves, leaf length, and leaf fresh weight, as compared to the control at all levels. This treatment also showed a significant increase in bulb and root fresh weight, root number, and root length. Application of SNP at 200 μM increased the values of the attributes studied to the maximum extent.

Key words: growth regulators, flower bulbs, pineapple lily, sodium nitroprusside

INTRODUCTION

Nitric oxide (NO) is a free radical and a signal molecule that plays an important role in plant signal transduction pathways (Baudouin & Hancock 2013). NO is present in plant tissues at very low concentrations, and its activity consists in stimulation or inhibition of various physiological and morphogenetic processes and hardening the plants against abiotic stresses (Sanz et al. 2015). The most common nitric-oxide-releasing compound (donor) is sodium nitroprusside (SNP) (Popova & Tuan 2010). Exogenous application of SNP was shown to break dormancy, stimulate seed germination and root system development, as well as affect growth and flowering (Liao et al. 2010; 2011; Gniazdowska et al. 2012; Niu et al. 2015; Wang et al. 2015; Arun et al. 2016).

Eucomis autumnalis (Mill.) Chitt. is an ornamental bulbous plant with huge potential on the horticultural market. The species belongs to Asparagaceae family and is endemic to South Africa (Zonneveld & Duncan 2010). *E. autumnalis* produces a very unusual inflorescence finished with a plume of leaves and comprising numerous white-green, sweet-scented flowers. It can also be grown as a pot or garden plant (De Hertogh & Le Nard 1993). As it produces anti-inflammatory and antispasmodic substances, *E. autumnalis* has been used in natural medicine for a long time (Zschocke et al. 2000). The main source of biologically active compounds are leaves, roots, and bulbs (Taylor & van Staden 2001; Masondo et al. 2014; Salachna et al. 2015a) that are collected from natural sites at a massive scale. This results in extinction of entire populations of *E. autumnalis*. Therefore, efficient methods of

*Corresponding author:
e-mail: piotr.salachna@zut.edu.pl

this species reproduction are sought after (Taylor & van Staden 2001; Ndhlala et al. 2012; Salachna et al. 2015b). The effects of various growth stimulants are also experimentally tested (Salachna & Zawadzińska 2015; Aremu et al. 2015; 2016).

To date, there have been no research reports on treating *E. autumnalis* with nitric oxide, which is why this study investigating the effects of various concentrations of SNP on morphological features of *E. autumnalis* was undertaken.

MATERIALS AND METHODS

The seeds of *E. autumnalis* were collected in the second half of September 2014 from the plants growing on experimental plots of the Department of Horticulture, West Pomeranian University of Technology in Szczecin (53°25′ N, 14°32′ E; 25 m.a.s.l.). Dry seeds were stored in paper bags in darkness at 18–20 °C (until January 15, 2015) and then at 4–5 °C (60 days). Following stratification, the seeds were disinfected with 50% ethanol, rinsed in distilled water, and sown into boxes filled with substrate TS 1 with pH 5.6 and EC 1.17 $mS \cdot cm^{-1}$ (Klasmann-Deilmann, Poland). The boxes were placed in a greenhouse with day/night temperature set at 22/18 °C. After 13 weeks, the seedlings, in batches of four, were transplanted into round plastic pots with a diameter of 16 cm (1.7 dm^3), which is filled with deacidified peat with pH 5.5 and EC 0.23 $mS \cdot cm^{-1}$ mixed with Yara Mila Complex fertilizer (Yara International ASA, Norway) containing 12% N, 4.8% P, and 15% K plus micronutrients, used at 2 $g \cdot dm^{-3}$. The pots were placed on a table in the greenhouse under natural photoperiod. SNP (Sigma-Aldrich, India) treatments were initiated 10 days after the transplantation. The plants were drenched with 150 ml per pot of SNP solutions four times, at weekly intervals, at the following concentrations: 0, 100, 200, 300, 400, and 500 μM SNP. Control plants were drenched with distilled water. On the last day of the experiment (September 21, 2015), leaf greenness index was determined in Soil–Plant Analysis Development (SPAD) units with an optical device Chlorophyll Meter SPAD–502 (Minolta, Japan). The measurements taken included three readings per each leaf. Then, the plants were removed from the pots, rinsed in tap water, and dried. After that, the following parameters were estimated: the number of leaves, length of the longest leaf, width of the longest leaf at its widest point, fresh weight of bulbs and roots, bulb diameter, and number and length of the longest roots. The experiment was designed as a completely randomized with 24 replications (6 pots × 4 plants per pot). The results were statistically analyzed by means of univariate analysis of variance and the post-hoc comparison were done using the Duncan multiple range test at P = 0.05 using STATISTICA 12.0 software.

RESULTS AND DISCUSSION

Exogenous application of SNP (a nitric oxide donor) had significant effects on leaf traits of *E. autumnalis* seedlings (Table 1). All SNP-treated plants had significantly higher greenness index of leaves up to 15.7–24.0% compared to the control. Among the different concentrations of SNP, treatment with SNP at 100, 200, and 500 μM concentrations had the greatest stimulatory effect on greenness index of leaves. These results are in agreement with those of Hayat et al. (2011) who reported that leaves of tomato plants treated with SNP at all concentrations (10^{-6}, 10^{-5}, or 10^{-4} M) had significantly higher greenness index (SPAD) over their respective controls, and the greatest increase was in leaves of plants from seeds treated with 10^{-5} M of SNP. Also, Beligni and Lamattina (2000) indicated that wheat seedlings sprayed with SNP and grown in darkness contained 30% to 40% more chlorophyll than control seedlings. A promotive effect of exogenous NO on chlorophyll content might perhaps be attributed to the role of NO in chlorophyll biosynthesis by enhancement of the conversion of Mg-protoporphyrin to protochlorophyllide and subsequently to chlorophyll (Abdel-Kader 2007).

The number of leaves per plant exhibited a dose-dependent effect to SNP (Table 1). Compared to the non-treated plants (control), the maximal number of leaves was obtained at 200 μM of SNP. Furthermore, application of SNP at 200 μM increased the leaf length, leaf width, and leaf fresh weight by 61.7%, 56.5%, and 308%, respectively (Table 1).

Table 1. Effect of sodium nitroprusside (SNP) at different concentration on leaf characteristics of *Eucomis autumnalis* seedlings

SNP concentration (μM)	Greenness index of leaves (SPAD)	No. of leaves	Leaf length (cm)	Leaf width (cm)	Leaf fresh weight (g)
0	31.2 ± 2.43 c*	2.75 ± 0.75 c	8.35 ± 0.87 d	1.47 ± 0.18 d	0.50 ± 0.11 d
100	38.5 ± 1.78 a	3.08 ± 0.51 bc	10.2 ± 0.65 c	1.68 ± 0.15 c	0.82 ± 0.14 c
200	38.7 ± 1.65 a	4.08 ± 0.51 a	13.5 ± 1.46 a	2.30 ± 0.28 a	2.04 ± 0.28 a
300	37.7 ± 3.05 ab	3.41 ± 0.51 b	12.5 ± 1.48 b	1.76 ± 0.29 bc	1.24 ± 0.20 b
400	36.1 ± 2.65 b	2.91 ± 0.79 bc	11.1 ± 1.22 c	1.65 ± 0.17 cd	0.93 ± 0.25 c
500	38.5 ± 1.68 a	3.25 ± 0.45 bc	10.7 ± 1.46 c	1.91 ± 0.29 b	0.78 ± 0.09 c

*Values are expressed as means ± standard deviation (n = 24). Values followed by a different letter in the same column were significantly different by Duncan's multiple range test at P = 0.05.

Table 2. Effect of sodium nitroprusside (SNP) at different concentration on root and bulb characteristics of *Eucomis autumnalis* seedlings

SNP concentration (μM)	No. of roots	Root fresh weight (g)	Root length (cm)	Bulb fresh weight (g)	Bulb diameter (mm)
0	3.91 ± 0.79 e*	0.08 ± 0.00 e	2.60 ± 0.24 c	0.30 ± 0.03 e	7.47 ± 0.45 c
100	4.91 ± 0.79 cd	0.37 ± 0.06 b	4.87 ± 0.45 b	0.56 ± 0.08 c	8.24 ± 0.42 b
200	6.58 ± 1.16 a	0.68 ± 0.11 a	6.87 ± 1.10 a	0.86 ± 0.06 a	9.06 ± 0.46 a
300	5.58 ± 0.99 bc	0.67 ± 0.08 a	4.81 ± 0.51 b	0.71 ± 0.13 b	8.49 ± 0.64 b
400	6.16 ± 0.71 ab	0.21 ± 0.05 c	4.96 ± 0.61 b	0.54 ± 0.07 c	8.15 ± 0.95 b
500	4.66 ± 0.77 d	0.14 ± 0.04 d	4.71 ± 0.62 b	0.39 ± 0.09 d	7.54 ± 0.49 c

*Values are expressed as means ± standard deviation (n = 24). Values followed by a different letter in the same column were significantly different by Duncan's multiple range test at P = 0.05.

In conformity with these results, An et al. (2005) reported significant enhancement in the length, width, and weight of leaf in maize seedlings as affected by the application of SNP. The authors found that exogenous NO was also able to elevate the activity of glucanases in leaf cell wall.

SNP treatments significantly increased all the examined root parameters of the seedlings compared to the control (Table 2). Of the tested dose of SNP, 200 μM proved the best. Compared with the control, the application of SNP at 200 μM increased the number of roots, root fresh weight, and root length by 68.3%, 750%, and 164%, respectively (Table 2). A similar positive effect of SNP at low concentrations on adventitious root initiation was observed in *Chrysanthemum morifolium* (Liao et al. 2010), *Tagetes erecta* (Liao et al. 2011), and *Petunia hybrida* (Arun et al. 2016). A stronger root system provides faster plant growth and faster production of bulbs of the commercial size, which ensures flowering.

SNP had significant effects on yield of bulb, and the effects were dose dependent (Table 2). From all SNP concentrations, 200 μM resulted in the greatest increase in bulb fresh weight and bulb diameter. Application of SNP at 200 μM enhanced the bulb fresh weight and bulb diameter by 186% and 21.3%, respectively, in comparison to the control. These are the first published results that indicate that NO molecules, when applied exogenously, positively improved bulbs yield. This positive effect of NO could be attributed to enhanced leaves and roots parameters (Tables 1 & 2).

This work strongly argues that the NO donor SNP when applied exogenously improved the growth of *E. autumnalis* seedlings. The effects of SNP was dose dependent, with the highest values of growth attributes response at 200 µM. All the examined growth parameters enhanced with SNP application might be ascribed to the role of NO in plant growth stimulation in general. However, physiological role of NO on plant growth and pharmacological properties of *E. autumnalis* is not yet known and will be the subject of future research.

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