EFFECT OF SODIUM NITROPRUSSIDE ON THE BIOCHEMICAL RESISTANCE OF MUNGBEAN TO COWPEA APHID

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Abstract: In this study, the inducible function of sodium nitroprusside (SNP, a nitric oxide donor) was evaluated based on the changes in some biochemical characteristics of mungbean (*Vigna radiata* (L.) Wilczek cv. DX208) involving resistance to cowpea aphid (*Aphis craccivora* Koch). SNP treatment was sufficient to increase content of chlorophylls, reduced aphid-infested damage, accumulated biosynthesis of antioxidants such as phenolic compounds and flavonoids in mungbean plants attacked by *A. craccivora*. Furthermore, SNP was also capable of either negatively altering the lifetime or diminishing fecundity of cowpea aphid. Those results demonstrated that SNP application improved the biochemical resistance of *V. radiata* cv. DX208 against its pest, *A. craccivora*.

I. INTRODUCTION

Nitric oxide (NO) donors, including sodium nitroprusside-SNP, are chemical inducers providing NO that has been known to trigger the specific defense responses in plants [1]. NO actually participates in several physiological processes and improves plants' tolerance of stresses [3, 7]. As a signaling molecule, NO is involved in defense enzymes/proteins (i.e., nitric oxide synthase, NO-associated protein 1, S-Nitrosoglutathione reductase), the acquired systemic resistance in plants [4], plays an important role in rice tolerance of *Nilaparvata lugens* feeding [5], pea defense response to *Acyrthosiphon pisum* [10], and tobacco defense against *Manduca sexta* attack [11].

Mungbean (*Vigna radiata* (L.) R. Wilczek) is an important legume crop that consumed as dry seeds and as bean sprouts. It serves as a cash crop for farmers and is an excellent source of digestible protein of low flatulence. Mungbean is popular plant among crops because of its short life cycle and drought tolerance, nitrogen fixation in its root nodules in association with soil *Rhizobia* allows it to thrive in N-deficient soils [12]. In the agricultural production in Nghe An Province, mungbean production is faced to many unfavourable agents, in which cowpea aphid (*Aphis craccivora* Koch) is one of the most critical. To date, information in the available literature regarding the defense mechanism of *V. radiata* against *A. craccivora* is still limited.

In the present study, the viability of SNP, an NO donor, was investigated through the change of some biochemical characteristics of *V. radiata* (L.) Wilczek cv. DX208 involving its resistance to attack of *A. craccivora* Koch.

II. MATERIALS AND METHODS

2.1. Materials

This study was performed on cultivar DX208 of mungbean (*Vigna radiata* (L.) Wilczek) plants, whose seeds have been supported by Nghe An Seed Center.

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Cowpea aphid (*Aphis craccivora* Koch) is cultured and supported by Department of Applied Entomology (Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Vietnam).

The NO donor used is solution of sodium nitroprusside-SNP (Merck, Germany).

2.2. Experiment

Mungbean seeds were surface-sterilized by $HgCl_2$ 0.01% for 10 minutes and then were imbibed in the incubator at 23^oC for 48 hours. Germinating seeds were cultured in 20-cm-diameter plastic pots containing Hoagland medium and placed in condition of 23±2^oC, the 70-75% RH, 110-130 $\mu M.m^{-2}.s^{-1}$ of light intensity and rhyme of 14 light /10 dark hours.

Mungbean plants at the V5 stage (five trifoliate leaves fully developed) were sprayed with 6 mL solution of 0.15 $g.L^{-1}$ SNP per plant. Two days after that, infestation of 30 individuals of cowpea aphid was started following formulae as:

(1) without SNP + without infestation of *A. craccivora* (control);

(2) without SNP + infestation of *A. craccivora* (- SNP + aphid);

(3) treating SNP + without infestation of *A. craccivora* (+SNP - aphid);

(4) treating SNP + infestation of *A. craccivora* (+ SNP + aphid).

All experiments were performed in Plant Physiology Labs (Vinh University) in years of 2016-2017.

2.3. Methods of analysis

a. The biological parameters of *A. craccivora* were assessed [8], including:

- Pre-reproduction, i.e., the length of 4-instar stages of cowpea aphid,
- Reproduction, i.e., a period of time by which a female adult can give birth to nymphs,
- Post-reproduction, i.e., the time of a female adult after having birth to die,
- Longevity, i.e., lifetime of each individual: from stage of 1st instars to death, and
- Fecundity, i.e., calculated as number of nymphs born by each female adult.

b. The tolerant indexes of mungbean plants were investigated on the 5th day after aphid application, including:

- Content of chlorophyll determined by Chlorophyll meter (model SPAD-502 Plus, Konica Minolta, Japan),
- Damage rate calculated by the electrolyte leakage assessment [9],
- Content of phenolic compounds analyzed by spectrophotometric method [6], and
- Content of flavonoids determined by the aluminum chloride colorimetric method [2].

c. Statistical analysis

All experiments were performed in three replicates. Significance tests and comparing treatments with the control for each experiment were evaluated by the one-way analysis of variance (ANOVA) at the 5% level. Data shown in the tables are means and standard errors (SE) for each variant.

III. RESULTS AND DISCUSSIONS

3.1. Effect of SNP on lifetime and fecundity of cowpea aphid

Treating SNP prolonged the length of *A. craccivora* pro-reproduction (*table 1*). This period time in the SNP-treated variant was 7.20 days which was 111.98% longer than the control (6.30 days). Contrary, SNP treatment resulted to shorten the pre-productive stage of cowpea aphid. The reduced time was about 11.40 days, having by 74.51% in comparison with the control (15.36 days). Similar to that, the post-reproduction stage of aphid was also cut down from 3.75 days to 2.68 days under effect of SNP. Total effects of SNP reduced lifetime of *A. craccivora* to 4.26 days.

Prolonging the pro-reproductive stage and shortening the reproductive and post-reproduction stages would negatively affect to reproduction of cowpea aphid. Treating SNP not only shortened lifetime but also strongly reduced quality of fecundity of *A. craccivora*. In mungbean plants as control, each female adult averagely gave birth of 89.53 nymphs in her lifetime, whereas in the formulae of treating by SNP, number of nymphs was 72.67, having by 81.17% in comparison with the control.

This result highlights the effect of SNP on limiting lifetime and fecundity of the *A. craccivora* population, and was similar previous studies [i.e., 10], which have been reported that application SNP may be an environmentally friendly option for postharvest pest control.

Table 1: *Effect of SNP on lifetime and fecundity of Aphis craccivora on mungbean DX208 plants*

Parameters / **Formulae**	**Pro-reproduction** *(days)*	**Reproduction** *(days)*	**Post-reproduction** *(days)*	**Lifetime** *(days)*	**Number of nymphs born per female adult**
Control	6.43 ± *0.74*	15.36 ± *1.27*	3.75 ± *0.55*	25.54 ± *3.18*	89.53 ± *6.28*
SNP	7.20 * ± *0.82*	11.40 * ± *0.97*	2.68 * ± *0.25*	21.28 * ± *1.51*	72.67 * ± *10.08*

(Data are expressed by mean ± SE of the three different replicates. In each column, (*) is considered statistically significant between the experimental variant and the control at the $P < 0.05$ level)

3.2. Effect of SNP on tolerance of mungbean DX208 plants

Aphid infestation remakably decreased content of chlorophylls. Under effect of 30 individuals of *A. craccivora*, content of both chlorophyll *a* and *b* in mungbean leaves obtained 0.382 and 0.137 $mg.g^{-1}$ FW, having by 77.86% and 88.39% of those recorded in the control plants, respectively (*table 2*).

Treating SNP resulted a difference in content of chlorophyll in different variants of mungbean. Levels of both chlorophyll *a* and *b* in mungbean without feeding of aphid were similar to control. On the contrary, those pigments in aphid-infested mungbean plants were 0.573 and 0.186 $mg.g^{-1}$ FW, 1.17 and 1.20-fold higher than those observed on control, respectively. It is probably that, in the unstressed enviroment, biosynthesis of chlorophylls in mungbean plants was not affected by SNP. However, under aphid infestation, content of chlorophylls in DX208 leaves was strongly accumulated by SNP to high level. This characteristics is very important for mungbean plants to improve photosynthesis, which supports effectively the essential materials and energy for them to cope with cowpea aphid attack.

The damage rate (based on electrolyte leakage and expressed as injury percentage) in mungbean DX208 leaves was different in the experimental variants. Infestation of *A. craccivora* resulted a serious damage to mungbean DX208 leaves without SNP. The injury rate was 15.93%, having 6.02-fold higher than that in the control (2.64%). SNP treatment expressed to reduce that damage. The injury percentage in the aphid-infested leaves was only 9.28%.

Without aphid infestation, content of total phenolic compounds in SNP-treated mungbean leaves (2.72 mg.g^{-1} FW) was statistically similar to that in the control (2.64 mg.g^{-1} FW). However, SNP treatment enhanced an increase in content of those antioxidants in the aphid-infested plants. A high amount of total phenolic compounds was obtained as 7.18 mg.g^{-1} FW, which was significant higher than that in variant without treating SNP (6.13 mg.g^{-1} FW).

Table 2: *The tolerant characteristics of mungbean DX208 plants*

Parameters / Formulae	**Content of chlorophyll** *(mg.g^{-1} FW)*		**Injury percentage** *(%)*	**Content of phenolic compounds** *(mg.g^{-1} FW)*	**Content of flavonoids** *(µg QE.g^{-1} DW)*
	chl a	**chl b**			
Control	0.490 ± *0.058*	0.155 ± *0.022*	2.64 ± *0.24*	4.61 ± *0.48*	39.16 ± *4.27*
- SNP + aphid	0.382 * ± *0.031*	0.137 * ± *0.019*	15.93 * ± *1.77*	6.13 * ± *0.57*	56.72 * ± 5.*41*
+ SNP - aphid	0.477 ns ± *0.066*	0.161 ns ± *0.024*	2.72 ns ± *0.41*	4.92 ns ± *0.48*	38.81 ns ± *5.06*
+ SNP + aphid	0.573 * ± *0.062*	0.186 * ± *0.025*	9.28 * ± *1.06*	7.18 * ± *0.97*	67.09 * ± *5.83*

(Data are expressed by mean ± SE of the three different replicates. In each column, (ns) is non-significant and (*) is considered statistically significant between the experimental variant and the control at the P < 0.05 level)

Similar to the phenolic compounds, a strong accumulation of flavonoids was recorded in *Vigna radiata* cv. DX208 plants attacked by *A. craccivora*. Level of flavonoids in aphid-infested leaves (56.72 µg QE.g^{-1} DW) was remarkably higher than that in the control (39.16 µg QE.g^{-1} DW). In addition, SNP treatment improved the biosynthesis of flavonoids in aphid-infested variants, content of those antioxidants was 67.09 µg QE.g^{-1} DW, having by 118% of that in variant without aphid infestation.

Previous studies presented that, the available descriptions of NO-mediated physiological processes are continuously increasing. Although it was initially associated with plant defense against pathogens, NO donor is also involved in plant responses to insects, including aphids. NO generated from SNP induced the defense enzymes, which was involved the phytohormonal signaling of salicylic acid, jasmonic acid, ethylene or connected to the defensive processes such as hypersensitive response and acquired systemic resistance in plants [4, 5, 10, 11]. In agreement with the above evaluations, our results suggested that SNP treatment improved the mungbean resistance to attack from cowpea aphid.

CONCLUSION

Application of SNP (an NO donor) was negatively altering the lifetime and diminishing fecundity of *A. craccivora* population. SNP treatment was also sufficient to increase content of the chlorophylls, reduced damage from aphid infestation, accumulated content of defensively bioactive compounds such as phenolic compounds and flavonoids in mungbean plants attacked by cowpea aphid. It is possible to suggest that SNP improved the biochemical resistance of *V. radiata* cv. DX208 against its pest, *A. craccivora*.

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TÓM TẮT

ẢNH HƯỞNG CỦA SODIUM NITROPRUSSIDE ĐỐI VỚI TÍNH CHỐNG CHỊU RỆP HẠI CỦA CÂY ĐẬU XANH

Nghiên cứu này đánh giá ảnh hưởng của sodium nitroprusside (SNP - một chất cung cấp nitric oxit) lên tính chống chịu của cây đậu xanh (*Vigna radiata* (L.) Wilczek cv. ĐX208) đối với tác động của rệp muội đen (*Aphis craccivora* Koch). Bổ sung SNP cho đậu xanh ĐX208 bằng cách phun lên lá đã gia tăng hàm lượng diệp lục, giảm mức độ tổn thương do rệp gây ra, đồng thời cảm ứng sinh tổng hợp các hợp chất phenol, flavonoit là những chất có vai trò quan trọng trong cơ chế tự bảo vệ của cây. SNP cũng trực tiếp tác động đến các pha sinh trưởng, phát triển, rút ngắn thời gian sống và làm giảm sức sinh sản của rệp. Những kết quả đó cho thấy SNP đã góp phần tăng cường tính chống chịu của cây đậu xanh ĐX208 đối với rệp muội đen.