ACCUMULATION OF FLAVONOIDS IN SOYBEAN UNDER EFFECTS OF CYANOBACTERIAL CRUDE EXTRACT AND APHID INFESTATION

Mai Van Chung1,*, Doan Manh Dung2, Do Ngoc Dai3

1Vinh University, 182 Le Duan, Vinh city, Nghe An province
2Hue University of Science, 77 Nguyen Hue, Hue city, Thua Thien Hue province
3Nghe An College of Economics, 51 Ly Tu Trong, Vinh city, Nghe An province

*Email: chung.uni@gmail.com

Received: 15 June 2016; Accepted for publication: 15 October 2016

ABSTRACT

The differential accumulation of endogenous flavonoids was recorded in soybean (G. max (L.) Merr. cv. “Nam Dan”) under infestation of cowpea aphid (Aphis craccivora Koch) and/or treatment of the crude extract of cyanobacterium strain Nostoc calcicola HN9-1a. During A. craccivora infestation, flavonoids in the aphid-infested leaves were strongly induced to high contents, which were 1.84–2.21 - fold higher than that observed in control. Those bioactive substances were suppressed by the single treatment of N. calcicola crude extract, however, were enhanced by the cross-talk interactions of N. calcicola HN9-1a and A. craccivora. Flavonoids in the double factors-effected soybean leaves were remarkably increased to high level since 24 hours after cowpea aphid attack, having by 2.99 - 3.06-fold higher than that in control. It was the important evidences to suggest that flavonoids may function in the defense mechanism of soybean “Nam Dan” against A. craccivora; and N. cacicola HN9-1a crude extract improved the accumulation of flavonoids in soybean response to cowpea aphid infestation.

Keywords: flavonoids, cyanobacteria, soybean “Nam Dan”, cowpea aphid.

1. INTRODUCTION

Cyanobacteria are known to produce various kinds of bioactive compounds that affect many physiological processes within living cells. Species belonging to genus Nostoc are regarded as good candidate for producing secondary substances that influence the growth of plants [1]. A large number of properties of Nostoc spp. have been identified as cytotoxic, antifungal, antibacterial, antiviral, immunosuppressive, enzyme inhibiting activities in the effect on higher plants [2]. Extract of some Nostoc species induces oxidative stress in plant cells by reactive oxygen species (ROS) productions resulting in lipid peroxidation and massive cell death as well as activating enzymatic antioxidants [3]. In our recent publication mentioned about the effect of strain N. calcicola HN9 on the antioxidant system of soybean “Nam Dan” (Glycine max
Mai Van Chung, Doan Manh Dung, Do Ngoc Dai

We revealed that, *N. calcicola* HN9 in death phase resulted oxidative stress and enhanced activity of antioxidant enzymes such as superoxide dismutases (SOD), catalase (CAT), ascorbate peroxidases (APX) and polyphenol oxidase (PPO) in soybean leaves [4]. Effects of *Nostoc* extract on plant defense mechanism, however, remain poorly understood. A number of chemotypes of flavonoids, including flavones, isoflavonoids, flavonoid-glycosides, have been known to protect crops against insects herbivory [5]. In legumes, these compounds reduce plants’ nutritive value, decrease digestibility of insects [6], or function as preformed or inducible anti-insecticidal properties [7], evenly act as toxins to pests [8]. Flavonoids also scavenge the free radicals, including ROS productions, and control their formation in living cells, therefore, reduce the oxidative damages [9].

To date, the available information of the insecticidal properties towards aphis of flavonoids in soybean plants is limited. In this study, we focused on the biochemical responses of *Glycine max* (L.) Merr. cv. “Nam Dan” to cowpea aphid-*Aphis craccivora* Koch (Hemiptera: Aphididae) concerning the expression of endogenous flavonoids. To partly assess the role of cyanobacteria on biosynthesis of flavonoids in soybean defense mechanism, we analyzed the changes of flavonoids’ content in *G. max* cv. “Nam Dan” leaves treated by the crude extract of strain *Nostoc calcicola* HN9-1a, which is hypothesized as an elicitor to soybean responses to aphid herbivores.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant

Plant used in the experiments is cultivar “Nam Dan” of soybean (*Glycine max* (L.) Merr.). Soybean seeds have been exclusively provided by Nam Dan Agricultural Extension Center (Vietnam). Soybean plants were cultured in 20-cm-diameter plastic pots containing Hoagland medium placed in the phytotron with temperature of 23 - 25°C, related humidity of 70 – 75 %, light intensity of 110 - 130 μM photons.m⁻².s⁻¹ and light period of 12 light/12 dark hours in the Plant physiology lab, Vinh University.

2.1.2. Aphid

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).

2.1.3. Cyanobacteria culture and extraction

The cyanobacterium strain, *Nostoc calcicola* HN9-1a, was collected from the rice field in Hung Nguyen district (Nghe An province) and isolated and cultured in the Phycology lab (Vinh University). *Nostoc* cells were cultivated in BG11 medium, pH 6.5 at temperature of 32 ± 2°C, under daylight fluorescent lamps, light period of 14 light/10 dark [3].

The *N. calcicola* HN9-1a biomass harvested in the stationary phase were centrifuged at 5,000 × *g* for 20 min and subsequently dried at 50 °C for 72 hours. The dried cells were ground to powder and extracted with 80 % methanol for 24 hours. The solution was centrifuged by 10,000 × *g* in 15 min to collect supernatant that was evaporated to obtain a crude brown gum [3].
2.2. Experiment

The cyanobacterial gum was dissolved in the distilled water at 0.00, 0.03 % and 0.05 %. These solutions were separately sprayed in leaves of soybean “Nam Dan” when plants were in stage V3 (three fully unrolled trifoliolates). After spraying 24 hours, each soybean plant was treated by 10, 20 or 30 wingless adults of *A. craccivora*. The control was soybean plants without aphid infestation. All variants were separately put in glass boxes (50 cm × 50 cm × 50 cm) covered by nylon gauze and placed in the phytotron with the environmental factors such as temperature, relative humidity, light intensity and light period controlled strictly.

Leaves of soybean plants were collected after 0, 24, 48, 72 and 96 hours post-infestation (hpi) of cowpea aphid. After all aphid individuals were carefully removed, leaves were weighed, frozen in nitrogen liquid and kept at -70 °C for subsequent analyses of flavonoids.

2.3. Analysis

2.3.1. Chemicals

All analytical chemicals were purchased from Singapore supplier of Sigma-Aldrich (USA).

2.3.2. Analysis of total flavonoids in soybean leaves

Total of 0.50 g frozen soybean leaves was extracted with 10 mL of 99.5 % ethanol under 200 rpm shaking for 24 hours. After filtration, the filtrate was adjusted to 10 mL with 80 % ethanol and centrifuged at 10,000 × g for 10 min at 4 °C, the supernatant was collected and the precipitate was then extracted with 5 mL of 80 % ethanol twice. Finally, the supernatant was combined with previous supernatant and adjusted to 20 mL with 80 % ethanol for analyses [10].

Content of total flavonoids in soybean leaves extract was determined by the aluminum chloride colorimetric method [10]. A mixture of 200 µL extract and 150 µL of sodium nitrite (NaNO₂ 5 %, w/v), was firstly incubated for 6 min at room temperature. Next, 150 µL of aluminium chloride hexahydrate (AlCl₃.6H₂O 10 %, w/v) was added and incubated for 6 min at room temperature, then 1,000 µL NaOH (10 %, w/v) solution was added; total of 1,500 µL mixture was incubated at room temperature for 25 min. The absorbance was measured at λ = 510 nm in the spectrophotometer UV-Vis CARY 60 (Agilent, USA) connected with a computer installed the data analytical software Agilent Cary WinUV 5.0. The calibration curve was established using quercetin dissolved in 80 % ethanol and then diluted to 25, 50, 100 and 200 µg.mL⁻¹ as the standards. Total flavonoids was calculated from the calibration curve y = 0.186x - 0.79 (the correlation coefficient R² = 0.9965) and were expressed in microgram quercetin equivalent per gram dry matter (µg QE.g⁻¹ dw).

2.3.4. Statistical analysis

All analyses were performed in at least three replicates. Analysis of variance (ANOVA) was applied to verify whether means from independent experiments within each given variant were significant at level P < 0.05. Data shown in the figures are means and standard errors (s.e.).
3. RESULTS AND DISCUSSION

3.1. Infestation of cow pea aphid accumulated flavonoids in soybean leaves

Infestation of *A. craccivora* accumulated generation of flavonoids in *G. max* (L.) Merr. cv. “Nam Dan”. In aphid-infested leaves, levels of flavonoids were immediately increased and were always higher than in the control during experiment (*Fig. 1*). The highest content of flavonoids was 149.32 μg QE.g⁻¹ dw in 30 aphid-infested leaves at 72 hpi, which was by 2.21- and 1.84-fold higher than at the beginning of experiments and in control plants, respectively. The high content of flavonoids in soybean “Nam Dan” leaves was resulted from the high infestation intensity from cowpea aphid. The significant differences between flavonoids levels in aphid-infested variants and control were recorded from 48-96 hpi (P < 0.05).

![Image of Graph](https://via.placeholder.com/150)

*Figure 1.* Generation of total flavonoids in leaves of *G. max* cv. “Nam Dan” control and *A. craccivora* infested leaves without treating Cyanobacteria.

Previous studies suggested a positive relationship between flavonoid content and the resistance/susceptibility characteristics against aphids of leguminous plants [11], whereby, the resistant lines showed the high accumulation of flavonoid under aphid effects. A strong generation of flavonoids in *G. max* cv. “Nam Dan” after *A. craccivora* infestation is an important evidence to suggest that, this cultivar maybe the resistant cultivar to cowpea aphid.

3.2. Cyanobacterial crude extract suppressed biosynthesis of flavonoids in soybean leaves

![Image of Graph](https://via.placeholder.com/150)

*Figure 2.* Effect of *N. calcicola* HN9-1a crude extract to content of flavonoids in *G. max* cv. “Nam Dan” without infestation of cowpea aphid.
Accumulation of flavonoids in soybean under effect of …

Crude extract of *N. calcicola* HN9-1a expressed to suppress biosynthesis of flavonoids in leaves of soybean “Nam Dan” (Fig. 2). Both two concentrations of extract such as 0.03 % and 0.05 % trended to reduce generation of flavonoids. Content of flavonoids in leaves treated by *N. calcicola* HN9-1a continuously decreased since 24 hpi and were significantly lower than in control within 48-96 hpi (P < 0.05). The lowest content of flavonoids recorded in soybean leaves treated by 0.05 % extract at 96 hpi was 41.75 μg QE.g⁻¹ dw, having by 54.23 % in comparing with control at the same point of time.

Flavonoids are often generated strongly when plants faced to stresses [9]. However, lack of information from available documents regarded the effected mechanism of cyanobacteria and their extract on biosynthesis of flavonoids in higher plants. This important aspect of the Cyanobacteria-soybean interaction should be clarified in the prospective studies.

3.3. Cyanobacterial crude extract improved the accumulation of flavonoids in soybean leaves infested by cowpea aphid

The cyanobacterial crude extract of *N. calcicola* HN9-1a reduced flavonoids’ levels in leaves of *G. max* cv. “Nam Dan” (Fig. 2), but it seems to improve the accumulation of flavonoids in soybean leaves after aphid infestation. Content of flavonoids in all aphid-infested soybean plants treated by *N. cacicola* extract was enhanced and remarkably increased to high levels within 24-96 hpi, which were significant higher than in control (P < 0.05) (Fig. 3).

![](image)

**Figure 3.** Content of flavonoids in leaves of *G. max* cv. “Nam Dan” under infestation of *A. craccivora* and treatment of *N. calcicola* HN9-1a extract solution 0.03 % (a) and 0.05 % (b).

The 0.03 % *N. calcicola* HN9-1a extract induced flavonoids in the infested soybean leaves strongly reached to peak within 48-72 hpi. The highest content of flavonoids obtained in the 20 aphid-infested leaves at 48 hpi was 207.79 μg QE.g⁻¹ dw, having by 2.99- and 3.06- fold higher than that observed in beginning and in control, respectively (Fig. 3a).

Denoting the changing in content similar to effect of 0.03 % *N. calcicola* extract, however, flavonoids in soybean leaves treated by 0.05 % concentration reached to maximum levels later, was within 72 hpi, and the highest level was resulted by infestation of 30 aphid individuals; whereas that in the 0.03 %-treated variant was 20 aphids (Fig. 3b).
In summarizing, generation of flavonoids in soybean “Nam Dan” under the cross-talk interaction of \textit{N. calcicola} HN9-1a extract and \textit{A. craccivora} infestation was early and remarkably accumulated. Their content was much higher than that observed in variants infested by cowpea aphid only. The crude extract of \textit{N. calcicola} HN9-1a may elevate the accumulation of flavonoids in leaves of soybean “Nam Dan” under infestation of \textit{A. craccivora}.

4. CONCLUSION

The high efficiency of flavonoids in regulation of ROS products has been known to constitute important lines of defense mechanism protecting plants against oxidative damages caused by infestation of aphids [4, 8]. The accumulated levels of flavonoids in \textit{A. craccivora}-infested leaves of \textit{G. max} cv. “Nam Dan” acted as a potential defense against cowpea aphid, while the crude extract of \textit{N. calcicola} HN9-1a additionally enhanced the accumulation of those antioxidants; under effect of cyanobacteria, flavonoids in the aphid-infested leaves was remarkably increased to high level. It was suggested that flavonoids might be a vital element in the defense mechanism of soybean “Nam Dan”, and \textit{N. cacicola} HN9-1a crude extract seemed to improve function of flavonoids in soybean response to infestation of cowpea aphid.

Acknowledgement: This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number: 106-NN.03-2014.22.

REFERENCES

Accumulation of flavonoids in soybean under effect of …


TÓM TAT

SINH TÔNG HỢP CỦA FLAVONOID Ở ĐẬU TƯỜNG DƯỚI TÁC ĐỘNG CỦA DỊCH CHIẾT VI KHUấn LAM VÀ RỆP HẢI

Mai Văn Chung1,*, Đoàn Mạnh Dũng2, Đỗ Ngọc Đại3

1Trường Đại học Vinh, 182 Lê Duẩn, Tp. Vinh, Nghệ An
2Trường Đại học Khoa học-Dại học Huế, 77 Nguyễn Huệ, Tp. Huế, Thừa Thiên Huế
3Trường Đại học Kinh tế Nghệ An, 51 Lý Tự Trọng, Tp. Vinh, Nghệ An

*Email: chung.uni@gmail.com

Dịch chiết vi khuẩn lam Nostoc calcicola HN9-1a và rệp muỗi den (Aphis craccivora Koch) đã cảm ứng khác nhau đối với sinh tổng hợp flavonoid trong lá đậu tương (Glycine max (L.) Merr. cv. “Nam Đàn”). Dựng tác động của rệp muỗi den, flavonoid trong lá đậu tương “Nam Đàn” đã cảm ứng tăng cao 1,84 - 2,21 lần so với đối chứng, và lượng flavonoid sinh ra tỷ lệ thuận với mức độ tác động của rệp. Tác động riêng của dịch chiết vi khuẩn lam N. calcicola HN9-1a không cảm ứng đối với flavonoids nhưng trong sự tương tác với rệp hại, đã kích thích tổng hợp chất chống oxy hóa này mạnh hơn 2,99 - 3,06 lần so với đối chứng. Cảm ứng sinh tổng hợp flavonoid là một phần ứng đáp trả của cây đậu tương “Nam Đàn” đối với sự phá hại của rệp muỗi den; và dịch chiết N. calcicola HN9-1a đã có tác dụng tăng cường vai trò của flavonoid trong cơ chế tự bảo vệ của giống đậu tương này đối với A. craccivora.

Từ khóa: vi khuẩn lam, đậu tương “Nam Đàn”, rệp muỗi den, flavonoid.