Science of the Total Environment 599–600 (2017) 198–206

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Salinity affects metabolomic profiles of different trophic levels in a food chain

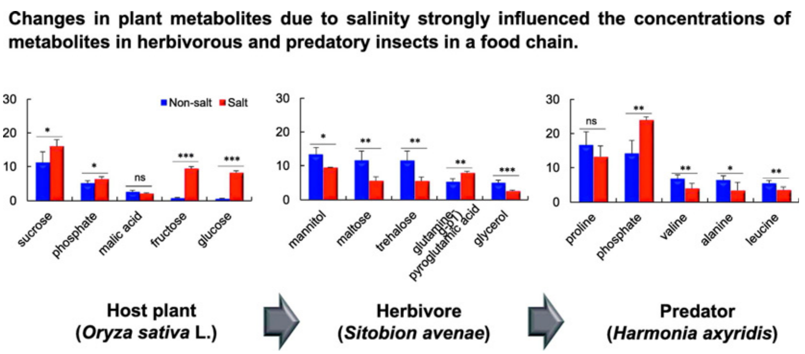
Kyong-Hee Nam [a,1], Young-Joong Kim [a,b,1], Ye Seul Moon [a], In-Soon Pack [a], Chang-Gi Kim [a,*]

[a] *Bio-Evaluation Center, Korea Research Institute of Bioscience & Biotechnology, Cheongju 28116, Republic of Korea*
[b] *Entomology Program, Department of Agricultural Biotechnology, Seoul National University, Seoul 08826, Republic of Korea*

HIGHLIGHTS

- We tested the effect of salt on metabolites in a plant–herbivore–predator system.
- Differences in metabolites between trophic levels or salt conditions were found.
- Salinity significantly increased the levels of main components in rice plant.
- In contrast, levels of major components in herbivores and predators were reduced.
- These might be correlated with inhibitive effects on insect growth under salinity.

GRAPHICAL ABSTRACT



Changes in plant metabolites due to salinity strongly influenced the concentrations of metabolites in herbivorous and predatory insects in a food chain.

Changes in plant metabolites due to salinity strongly influenced the concentrations of metabolites in herbivorous and predatory insects in a food chain.

ARTICLE INFO

Article history:
Received 6 March 2017
Received in revised form 27 April 2017
Accepted 1 May 2017
Available online 3 May 2017

Editor: D. Barcelo

Keywords:
Food chain
Harmonia axyridis (multicolored Asian lady beetle)
Metabolomics
Rice (*Oryza sativa* L.)
Salinity
Sitobion avenae (wheat aphid)

ABSTRACT

Salinization is one of the most important abiotic stressors in an ecosystem. To examine how exposing a host plant to excess salt affects the consequent performance and metabolism of insects in a food chain, we determined the life history traits and the metabolite profiles in rice (*Oryza sativa*), the herbivore *Sitobion avenae*, and its predator *Harmonia axyridis*. When compared with performance under normal (non-stressed) conditions, exposing plants to 50 mM NaCl significantly delayed the timing of development for *S. avenae* fed on rice and *H. axyridis* and also reduced the body mass of the latter. Our GC–MS-based analysis revealed clear differences in metabolite profiles between trophic levels or treatment conditions. Salinity apparently increased the levels of main components in rice, but decreased levels of major components in *S. avenae* and *H. axyridis*. In addition, 16 metabolites showed salinity-related contrasts in this trophic interaction for our rice–*S. avenae*–*H. axyridis* system. Salinity impeded the accumulation of metabolites, especially several sugars, amino acids, organic acids, and fatty acids in both insects, a response that was possibly associated with the negative impacts on their growth and reproduction under stress conditions.

Abbreviations: GC–MS, gas chromatography–mass spectrometry; ICP–OES, inductively coupled plasma–optical emission spectrometer; PCA, principal component analysis.

[*] Corresponding author.
E-mail address: cgkim@kribb.re.kr (C.-G. Kim).

[1] These two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.scitotenv.2017.05.003

1. Introduction

Salinization is one of the most important abiotic stressors within an ecosystem. Although salt stress originates from natural sources, increased salinization due to human activity is becoming a serious threat to biodiversity (Cañedo-Argüelles et al., 2013; Daliakopoulos et al., 2016). Salt stress can influence nutrient uptake, photosynthesis, and water relations in plants, thereby reducing their growth and productivity (Parida and Das, 2005). Furthermore, the performance and fecundity of many organisms at higher trophic levels in a food chain (i.e., herbivores and predators) can be affected when diverse biotic and abiotic stresses alter the chemistry of the host plant (Awmack and Leather, 2002; Ode, 2006). Limited nitrogen and water inputs to plants are negatively correlated with life-history traits and the foraging behavior of insect herbivores and their predators (Han et al., 2015, 2016a).

Albarracin and Stling (2006) have demonstrated the bottom-up effects on plant–insect interactions because of salinity stress. High application rates of NaCl spray can significantly elevate the concentration of carbohydrates and amino acids in the phloem exudates of hawthorn (*Crataegus* spp.), which then markedly stimulates a rise in nearby populations of the green apple aphid *Aphis pomi* (Braun and Flückiger, 1984). In contrast, increasing the proportion of NaCl in an irrigation solution can enhance glycine-betaine accumulations in wheat (*Triticum aestivum*) seedlings while greatly reducing the rate of population growth for the aphid *Schizaphis graminum* (Araya et al., 1991). Elevated soil salinity increases the production of cyanide-containing glycosides in *Trifolium repens*, which results in improved resistance against two natural herbivores, *Deroceras reticulatum* and *Hypera punctata* (Ballhorn and Elias, 2014). Increasing the salinity of the irrigation solution for tomato (*Solanum lycopersicum*) plants significantly elevates the production of tomatidine in their leaves, and also accelerates the rate of development by the leaf miner *Tuta absoluta* without lowering its pupal mass (Han et al., 2016b). However, little is known about the impact of salinity on tritrophic interactions among host plant, herbivore, and predator. Studies on salt-induced metabolic changes have generally focused on a single trophic level, mainly the plant, and few have examined the trophic relationship between producer and consumer.

Metabolomics is a useful approach for investigating the complex interacting mechanisms of cellular metabolic pathways in response to abiotic stresses (Fiehn, 2002). In particular, ecological metabolomics is the most appropriate way to comprehend the complete response of an organism to environmental changes, including exposure to environmental toxicants (Sardans et al., 2011; Fasulo et al., 2012; Cappello et al., 2013, 2016a, 2016b; Brandão et al., 2015). Several studies have considered the responses of some metabolic pathways in organisms to changes in abiotic factors such as cold, warming, drought, and salinity (Bundy et al., 2003; Johnson et al., 2003; Kaplan et al., 2004; Alvarez et al., 2008). Overgaard et al. (2007) have used metabolomic profiling via proton nuclear magnetic resonance spectroscopy to assess the response in *Drosophila melanogaster* during periods of rapid cold hardening and cold shock treatment. However, metabolomics analyses are rare with regard to the plant–insect interaction when organisms are subjected to abiotic stresses. Furthermore, no evaluations have been reported about the accumulation of salinity-induced metabolites among three trophic levels in plant–herbivore–predator systems. Miyagi et al. (2013) have suggested that understanding the host plant–predator relationship at the metabolite level will provide evolutional clues about how living organisms adapt to such environments.

Accordingly, we designed our experiments to ascertain the implications of salinity stress on ecological factors, such as the survival, growth, and reproduction of herbivorous and predatory insects, as well as on their metabolite profiles, by examining the tritrophic interactions within a plant–herbivore–predator system. For this, we set up a three-trophic-level food chain composed of the rice plant, *Oryza sativa* L.; the herbivore *Sitobion avenae*, and its predator *Harmonia axyridis*. These insects were selected because the wheat aphid *S. avenae* is relatively abundant, feeding mainly on members of the Poaceae family (Blackman and Eastop, 2000), and the multicolored Asian lady beetle *H. axyridis* is a well-known, natural enemy of various aphid species (Koch, 2003). In addition, we analyzed a comprehensive metabolite profiling in each of the trophic levels and in their interactions.

2. Materials and methods

2.1. Test species preparation

In September 2014, individuals of *Sitobion avenae* were collected in an experimental paddy field at the Korea Research Institute of Bioscience and Biotechnology (KRIBB), Cheongju, Chungcheongbuk-do, Korea (36°43′N, 127°26′E). A colony of this herbivore was then maintained on ‘Hwayoung’ rice plants in a greenhouse (day/night 28 °C/22 °C, 16-h photoperiod).

In January 2015, adults of *Harmonia axyridis* were collected from their hibernation site on Mock-ryeong Mountain in Cheongju, Chungcheongbuk-do, Korea. A colony of this predator was maintained in an insect rearing room (25 ± 1 °C, 50 ± 5% relative humidity, and 16-h photoperiod).

2.2. Monitoring the effects of salinity on the growth and development of S. avenae

Because soils are classified as salinity-affected when the electrical conductivity is 4 dS/m or higher (equivalent to approximately 40 mM NaCl; Munns and Tester, 2008), we subjected our host plants to saline conditions by adding 50 mM NaCl to the soil.

After rice seeds were sown in a tray filled with potting soil, the germinated seedlings were placed in a greenhouse. After 30 d of growth, each seedling was transplanted into a custom-made cylindrical transparent acrylic cage (95 mm diameter, 310 mm height) filled with 425 ml of potting soil. Each cage had seven holes (10 mm diameter) in the bottom for introducing liquids plus an acrylic cap with nylon mesh to provide ventilation. The seedlings were kept in an insect rearing room.

Ten replicated, caged seedlings were put in a plastic tray (550 mm × 350 mm × 150 mm), to which 26 l of either tap water or 50 mM NaCl solution was added through the holes. Soil salinity was determined using a multi-parameter water quality meter (WQC-24; DKK-TOA Co., Tokyo, Japan). A cohort of three first-instar nymphs was transferred to each plant. After 24 h, all remaining insects were removed except for one newborn first-instar nymph. For investigating the life history traits of *S. avenae*, the numbers of live or dead insects and their developmental times (based on the presence of exuvium) were recorded at 24-h intervals for 20 d. The intrinsic rate of increase in this population was calculated using the equation $r_m = 0.738$ ($\ln M_d/T$), where T is the number of days from birth to onset of reproduction and M_d is the number of offspring that reproduced during time period T (Wyatt and White, 1977). New applications of tap water or NaCl solution were made every 3 d.

2.3. Monitoring the effects of salinity on the growth and development of H. axyridis

Rice seeds were sown in a tray. After 20 d of growth in a greenhouse, individual seedlings were transplanted into 1/5000 a Wagner pots filled with potting soil. The pots were arranged on five benches in a randomized complete block design with 80 replicates. Each block contained 16 replicates of the stress treatment, i.e., salt (800 ml of 50 mM NaCl solution) versus non-salt (800 ml of tap water). These treatments were applied at 7-d intervals. After 6 weeks, 10 randomly selected adults of *S. avenae* were transferred to each plant and a colony was proliferated to provide an adequate food resource for *H. axyridis*.