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Aphid–plant interactions: How saliva of aphids modulates the plant responses

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Abstract

Aphids are one of the most important herbivore pests of agricultural and forest crops. They have specialized long stylets adapted for the sucking of sap from the phloem. To successfully feed on the host plants aphid need to counter the physical and chemical defense system of plants. The present study is conducted for the first time to my best knowledge. In this study, *Acyrthosiphon pisum* was reared on two-week-old *Pisum sativum* after that the adult aphids were transferred to the lab and were fed on the artificial diet to collect the saliva of both types. The Aphid species were inoculated with *Buchnera aphidicola* and *Regiella insecticola* to study the impact on saliva composition and plants' response toward these strains. The results indicate the aphid inoculated with *B. aphidicola* had a long and thick salivary flange and also they resist the plants to develop occlusion to prevent the loss of sap from Phloem. In addition, the aphid needs to bypass the other plant defenses; aphid has been shown to affect the plant metabolism.

Keywords: plant-aphid interaction, phloem tube occlusion, Aphid saliva, aphid artificial diet

Introduction

Aphids are considered as one of the major groups of herbivores belonging to the order Hemiptera and the family Aphididae exclusively phloem feeder distributed throughout the world. They cause serious losses to cultivated crops as the crop pest aphid affects the economy by excessive feeding on phloem sap and also as a major vector of some important viruses. Out of 5000 well-described aphid species, 250 species are considered important agricultural pests worldwide (Blackman & Eastop, 1994; Blackman & Eastop, 2000; Kumar, 2019) [1-3]. As the aphid can adapt to every environmental condition, so this ability makes it a cosmopolitan pest of agricultural pest (Poirié & Coustau, 2011) [4]. The ability to limit productivity is just because of its biology and population strategies. These herbivore insects have long cylindrical mouthparts which are used to pierce the tender plants and suck the sap (sugar and nutrients) from the phloem. This specific feeding behavior is supported by a high reproductive rate. Aphids show the parthenogenesis and viviparity for reproduction. Aphids are exclusively phloem feeders distributed throughout the world. They cause serious losses to crops including agronomic crops. The higher population densities lead to the withdrawal of a large number of nutrients from the sieve tubes and also the transfer of a large number of different viruses.

Aphids ingest sap through piercing-sucking mouthparts also known as a stylet. In most cases, the aphids exhibit passive feeding by high pressure in sieve elements and feed on different plant species. Most of the species are specialist feeders and prefer to feed on a single host plant while some of them have a broader host range (Peccoud J, *et al.*, 2010**)** [5]. Heteroecious aphid species live on one plant species during winters and move to other plant species during summer and again migrate to other plant species in autumn. During feeding aphids' stylets puncture the epidermal, mesophyll, and parenchyma cells and this damage may influence the plant response to the aphid infestation **(**Williams IS, *et al*., 2007**)** [6]. Girousse *et al.* recorded the similarities between the effects of pea aphid (*Acyrthosiphon Pisum*) on alfalfa and also the effect of thigmotropism on the reduction of stem length. Aphids penetrate the plant tissues through the intercellular route and their impact on the host plant is studied through due to withdrawal of sap and injection of the saliva in the host plant. Aphids usually secrete proteinaceous saliva sheath that lines the stylet pathway and also water saliva that contains large numbers of enzymes such as pectinases, cellulases, and oxidases (Forbes AR, 1977) [8]. Williams IS *et al.*, (2007) [6] recently conducted a study and showed that the saliva of aphids also contains calcium-binding proteins that can reverse the phloem occlusion triggered by the calcium flux in response to the wounding caused by the aphid. The ability of the aphid to stop blocking sieve tubes is an important adaptation that allows the aphid to suck the sap and remained there for several hours. As the sap is an unbalanced diet and is primarily composed of sugars and amino acids with a higher percentage of carbon and nitrogen. To overcome the effect of this excessive amount of sugar content aphids have developed the modifications in their intestinal tract and filter out the extra sugar in the form of honeydew **(**Dixon AFG, 1998**)** [10]. The amino acid contents are in very low concentration, but despite their low nutrition aphid exhibit a higher growth rate and also a higher reproduction rate. The essential amino acids required by the aphid for their growth and development are synthesized by symbiotic bacteria. There are two types of bacteria associated with aphids: the primary symbionts (obligate) and secondary symbionts (facultative). *Buchnera aphidicola* and *Escherichia coli* are the members' primary symbionts while *symbiotic* and *Regiella insecticola* are the members of secondary symbionts (Munson MA, *et al.*, 1991; Loudit SMB, *et al.*, 2018) [11, 12]. These

symbiotic bacteria upgrade the diet by converting the non-essential amino acids into essential amino acids.

The present study was designed to separate the saliva of different aphid species having different endosymbiotic bacteria and also how these endosymbionts elicit the plant defense mechanism.

Materials and Methods

Aphid and plant breeding

The pea aphid *Acyrthosiphon pisum* was reared on two week old *Pisum sativum* major host plant in Perspex cages with a large window on one side to place and remove plants. The boxes were placed under controlled conditions in the lab and the regime was set at an average temperature of 20-25ºC. The plants were cultivated in a greenhouse with an average temperature of 20±2 and in natural cold lights under an L8:D16 period (SONT Agro 400 W, Phillips, Eindhoven, and The Netherlands). The aphid species were used for the experiments and the chemical composition of saliva was also tested. The scanning electron microscope was used to study the salivary flange and the aphid was on the leaf during the SEM preparation.

Saliva collection chambers

About 1,500-2,000 aphids were collected from the plants and were poured directly onto the upper surface of the Parafilm of the saliva collection chamber filled with an artificial diet of different compositions Williams IS, *et al.*, (2007) [6]. The saliva collection chamber is a Perspex block that has a shallow bath of about 10cm radius and has a depth of about 1mm. The collection chamber was sterilized and filled with about 3ml of artificial diet. Aphid penetrated the Parafilm with their stylets to feed the artificial diet. As the saliva consists of both insoluble and soluble parts, so the insoluble saliva compounds are deposited on the Parafilm and the soluble compound secreted into the artificial diet were recovered separately after 24hrs of placing artificial diets in the chambers.

Diets for aphid saliva collection

The collect the saliva of aphids two basic diets were used for the collection of gel and water saliva the conditions were mimicked a) sieve-elements (SE) the sap (15% sucrose, 100 mM L-aspartic acid with a pH of 7.2 (KOH), 100 mM L-methionine and 100 mM L-serine and (Williams IS, *et al.*, 2007; Giordanengo P, *et al.*, 2010) [6, 13] and b) cell-wall (CW) milieu (10 mM MES, 1 mM CaCl2, 20 mM KCl and adjusted to pH 5.5 (KOH) (Thomma BPHJ, *et al.*, 2001) [14]. Before the use, the diets were sterile with the help of RotilaboH syringe filters (Carl Roth GmbH, Karlsruhe, Germany) with a pore size of 0.45mm.

Response of plants to aphid attack

To obtain the images of the response of plants towards aphid attack, leaf sections of about 5×5mm, which were previously colonized by the aphid are clipped off from the cages after 7 days. The leaves were dehydrated in ethanol with varying concentrations 60; 80; 90 and 95%) and finally placed in acetone which had the silica gel to remove the maximum water. The leaves were attached with the electron microscopy specimen holders with the help of carbon glue. Phloem structure was compared with the control where no aphid attack was recorded.

Results

Salivary flange during and after stylet penetration

As the aphid saliva is gel type also so it is secreted at the stylet at the penetration site on the Parafilm sheath and forms the flange that is present around the tip of the stylet tip (Table 1). After the removal of the stylet, the flanges remain there on the Parafilm sheath and appear to be plugged by gel saliva. Sometimes in the present study, the gel saliva appears just like bubbles, which indicates the release of a large amount of saliva. The artificial diet analysis indicates that watery saliva was also recorded before feeding.

Table 1: Comparison of sheath formation the saliva proteins and aphid settling on various diets

Diet	No. aphids feeding after 12hrs	No. sheaths/aphid	Water saliva proteins
Pure water	3.2±0.63	1.67±0.53	0.051±0.03
15% sucrose	6.4±0.84	1.89±0.23	0.89±0.08
Amino acid diet	3.8±0.67	1.83±0.43	0.18±0.02

Response of plants to aphid attack

The plants have developed the response against mechanical damage; plants immediately occlude the injured areas of sieve elements just to prevent the loss of sap. The occlusions may be callose (a b-1,3 glucan polymer) formation, constricting the pores by plugging in the phloem-related proteins. These types of proteins were only recorded in the case of plants with aphid attacks while these proteins were not recorded in the case of control plants. The study indicates that within 24 hrs the plants' phloem cells induce occlusion after injury, while the stylet penetration does not affect the sap flow. The gel saliva sheath maybe seals the sieve element to minimize the loss of sap from the tubes.

Discussion

The aphids secrete saliva of different types under symbiotic relationships with different bacteria. As the aphid secretes two types of saliva the gel and the watery, the gel saliva is secreted during the penetration of stylet and watery saliva is secreted during the sucking of sap. The secretion of gel saliva starts with the formation of the salivary flange on the surface of the plant part. The salivary flange prevents the stylet from slipping away during the epidermal piercing (Razaq A, *et al.*, 2000) [15]. The hole inside the stylet canal is sealed immediately after stylet removal because no hole appeared in the flange. The salivary sheath is formed with the propagation of stylet inside the plant parts and protects the stylet. Under SEM the sheath of gel saliva appears as the necklace of pearls (Hewer A, *et al.*, 2011) [16].

The initial secretion of the gel saliva droplet is followed by the secretion of the watery saliva that inflates the droplets of the gel saliva and makes it a canal-like structure and cavity inside these droplets. This tubular structure is observed by SEM; these secretions harden after proposition and insertion of the stylet. The watery saliva is exclusively secreted sucking conditions while gel saliva is secreted during the penetration. The present study indicates that the watery saliva is not secreted until it reaches the diet with pH 7 as indicated in a previous study (Hewer A, *et al.*, 2010) [17].

The plant under aphid infestation responds in a variety of ways.

The feeding by aphids withdraws a large quantity of sap which leads to sometimes local area chlorosis and weakening of the plants and decreases plant resistance to different diseases and pathogens. Further, the saliva may also affect the hormonal balance of the plants which may lead to abnormal cell division and gall formation (Otha S, *et al.,* 2000) [18]. Plants responded by immediately making occlusion in sieve elements to prevent sap loss (Chen JQ, *et al.,* 1997) [19]. The seepage of the sap is prevented by callose formation and constricting of the sieve pores by phloem-related proteins (Chen JQ, *et al.,* 1997, Li Y *et al.,* 2007) [19, 20].

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