American Journal of Plant Sciences, **2013, 4, 47-51**
Published Online December 2013 (http://www.scirp.org/journal/ajps)
http://dx.doi.org/10.4236/ajps.2013.4.12A3006

Scientific Research

Expression Pattern of Sucrose Transporters in *Arabidopsis thaliana* during Aphid (*Myzus persicae*) Infestation

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Received September 22nd, 2013; revised November 18th, 2013; accepted December 10th, 2013

ABSTRACT

Herbivorous insects change the metabolism of the plant during their attack. Our study reports the changes in the expression pattern of sucrose transporters in response to the infestation of aphids at different time intervals. Results showed a significant enhancement in the expression pattern for six out of nine sucrose transporters in response to aphid infestation, followed by suppression after some point. During an earlier time point of infestation, the expressions of sucrose transporters were enhanced probably to compensate for the energy requirements of the damaged cell. However, suppression of sucrose transporters at a later stage may be a defense strategy of the plant to repel the aphids because at a later stage of infestation, aphids become a secondary sink. To complement our assumption, we performed aphid infestation choice and reproductive performance tests in the null mutant of one of the transporters, SUC2, which was compromised in phloem loading of sucrose. Results showed that the mutant was less preferable to aphid for choice as well as reproduction performance.

Keywords: Sap Sucking Insects; Aphid; Sucrose Transporters (SUCs)

1. Introduction

Among herbivorous insects, sap-sucking insects pose a serious problem for both crops as well as glasshouse plants [1]. By continuously sucking the sap, these insects not only become a sink in plants [1], but also spread viral diseases [2]. For their protection, plants have evolved pre-attack barriers, such as cell wall, cuticle, trichomes, proteinase inhibitors, and polyphenol oxidase, as well as post-attack weapons, such as attracting parasitoids [3]. Disaccharide sucrose is an inactive photosynthetic product which is produced in the photosynthetic parts of the plant and transported to the sink by several sucrose-H^+ symporters [4] and utilized by sap-sucking insects. Sucrose transporters (SUCs) are the chief mediators in carbon partitioning. In *Arabidopsis thaliana*, nine SUCs are reported. The expressions of SUC1 (AT1G71880) in pollen and root [5], SUC2 (AT1G22710) in companion cells [6], SUC3 (AT2G02860) in guard cells, trichomes, germinating pollen, root tips, the developing seed coat, and stipules [4], SUC4 (AT1G09960) in the sink tissue (developing leaves) and minor veins [7], SUC5 (AT1G 71890) in developing seeds, and SUC8 (At1g66570) and SUC9 (At5g06170) in floral tissues [8] are reported. However, the expression profiles of SUC6 (AT5G43610) and SUC7 (AT1G66570) have not been analyzed much and are mentioned as pseudo transporters [9]. All the SUCs are different in their kinetic properties, substrate specificity, and expression patterns. Therefore, the comparative expression profiling of the SUCs after aphids attack will be of significant interest because these transporters are exclusively related to phloem loading of sucrose and aphids attack exclusively on phloem. Though reports are available on the expression pattern of SUC3 [4] in response to wounding and SUC1 in response to nematode attack [10], a report on the expression pattern of SUCs in response to aphid attacks is still lacking. Therefore, in the present study, we have investigated the expression pattern of SUCs in *Arabidopsis thaliana* during aphid (*Myzus persicae*) infestation.

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2. Materials and Methods

2.1. Plant Growth and Aphid Collection

Plants of *Arabidopsis thaliana* (Col-0 ecotype) and SUC2 mutants (Col-0 background, SALK_038124) were grown on vermiculite and solarite soil. Seeds of both wild-type and mutants were placed at equal distance in 10" plastic pots and kept at 4˚C for 3 days, and then under a 16-h-light/8-h-dark condition at 22˚C. For RNA isolation and qRT-PCR, three plants were grown per pot. Twenty one-day-old plants were selected for the qRT-PCR experiment, infestation choice, and reproductive performance tests. Aphid culture was maintained on potted *A. thaliana* plant in the laboratory at 22˚C ± 2˚C and 70% relative humidity.

2.2. Aphid Infestation, RNA Isolation, cDNA Preparation, and qRT-PCR

Ten aphids per plant were released on 21-day-old plants and the RNA isolated using IHBT kits after 2 h, 24 h, 48 h, 72 h, and 96 h intervals of infestation. All the experiments were performed in triplicates. After qualitation and quantification, 10 µg RNA was used for DNase treatment (Ambion). DNase-treated RNA (2 µg) were used for the cDNA preparation using SuperScript® cDNA Synthesis Kit (Invitrogen). The quantitative reaction was performed on ABI 7500 Real-Time PCR Detection System (Applied Biosystems) using the SYBR Green PCR Master Mix (Applied Biosystems, CA, USA). The expressions of selected genes were normalized against an internal reference gene actin (AT3G18780.2) [11]. The primer sequences used in this study are given in **Table 1**.

2.3. Aphid Infestation Choice and Reproductive Performance Test

Twenty one-day-old Col-0 and mutant plants of equal sizes were subjected to aphid infestation. One hundred aphids were placed into the center of pots containing filter paper for the free movement of insects between the rows of 5 wild-type and 5 mutant plants. After 24 h and 72 h, the number of aphids on each plant (mutant and wild-type) was counted. The whole experiment was performed in duplicates. Means were analyzed by one-way ANOVA and compared using Duncan's Multiple Range Test. For reproductive performance, the set-up of plants was the same as that of the infestation choice test, except that four aphids (fourth instars) per plant were released. Population reduction in mutant as against Col-0 was calculated by the formula [12] of PROC (Population reduction over control in %) = $100 \times 1 - (Ta \times Cb)/(Tb \times Ca)$, where Ta = Population after treatment; Tb = Population before treatment; Ca = Population after control; Cb = Population before control.

3. Results

3.1. Expression Pattern of *A. thaliana* (Col-0) Sucrose Transporters (SUCs) in Response to Aphid Infestation

Significant changes in the SUCs expression were seen after two hours of infestation (**Figure 1**). The first induced expression of SUC6 (11 fold) was observed at 24 h of infestation followed by decrease in its expression during the time duration of infestation. SUC2 showed maximum expression at 48 h of infestation, followed by SUC6. However, the expression of SUC1 was maximum at 72 h of infestation (5.9 fold) and SUC3, SUC4, SUC7, and SUC9 showed poor inducibility. We also compared the qRT-PCR result with the publicly available data set (GSE5525) [13] of 48 h and 72 h aphid-infested *A. thaliana* microarray. The expressions of SUC1, SUC2, SUC3, SUC4, SUC5, and SUC7 were found only at 72 h, but only SUC1 showed significant induction (more than two-fold). The expression profile of SUCs in the Gene investigator showed highest expression of SUC2 and SUC1

Table 1. Primers sequence used in real time PCR.

	Forward primer (5' - 3')	Reverse primer (5' - 3')
SUC1	GGGTCGTCTGTATTTCACCC	CACACAAATCTTATTTAAGGGC
SUC2	CCGGAACGGCTTCGTAAGA	GATTCCGAGTAGCTGCACGTAAG
SUC3	CAAGAACCGCAGCCGTAATC	CTTGACCGCCACCGGAAT
SUC4	AGTGTCAAGCGAGGAACGCATA	AGTCACACGAGAAGCCATTGC
SUC5	GGGCTATGGGATTCCATTAG	TAAAAGACAGACGACCAAGG
SUC6	TCCTGTCTCCGGCCTGCTT	AGGCGCCCATAGCGATGA
SUC7	GTCTTTAAGAGACAAGCCCAC	AGACTGTCTATCCACAGTCGT
SUC8	CTAGCTTCCATAATCTCAAGT	TTGGTAAGTTTCCACCTCCAAAA
SUC9	GTGGTTCCTGATGAGCCG	GAGAAGCTGAACGTATGGG
Actin	TCCCTCAGCACATTCCAGCAGAT	AACGATTCCTGGACCTGCCTCATC

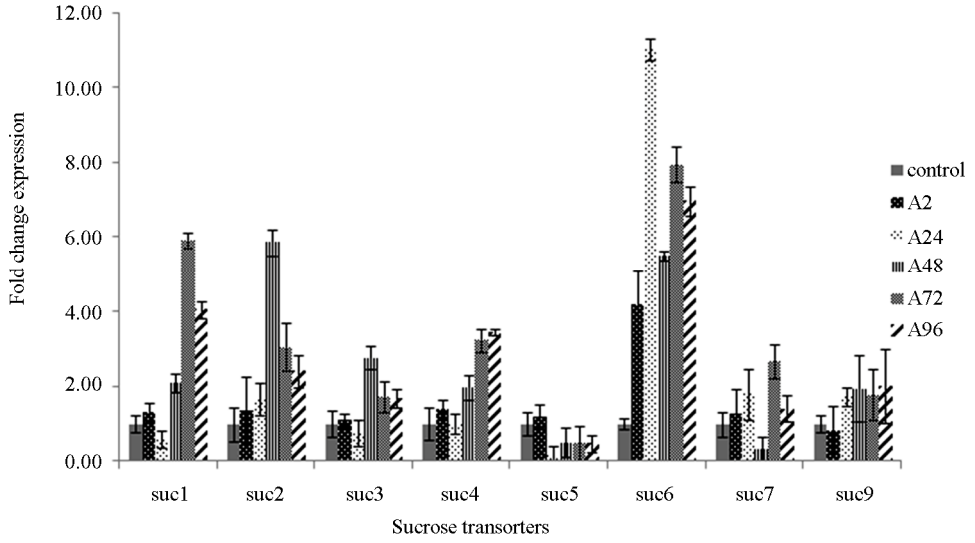

Figure 1. Expression pattern of sucrose transporters (SUCs) in different time points of aphid infestation (A2; A24; A48; A72, and A96 stand for aphid 2 h; 24 h; 48 h; 72 h; and 96 h of infestation).

in the rosette and cauline leaf, followed by SUC3, SUC4, SUC5 SUC6, and SUC9, and the least in SUC8. In our results, there was almost the same expression pattern obtained, except that SUC8 was absent and least expression was of SUC5 (**Figure 1**). Interestingly, the expression of SUC6 was absent in the microarray experiment, but it was the highest expressive in our observation after 24 h of aphid infestation.

3.2. Aphid Infestation Choice Test

SUC2 is exclusively expressed in companion cells [14] and is involved in the phloem loading of sucrose [6]. To see the infestation behavior of aphids between normal plants and plants with less sugar (in their sap), we selected wild-type and null mutants (SALK_038124) [15] of SUC2. Our results show that approximately three times more aphids were attracted toward the wild-type than the mutant (**Table 2**).

3.3. Aphid Reproduction Performance in SUC2 Mutant and Wild Col-0 Plants

The aphid reproduction performance was checked in the SUC2 mutant (SALK_038124) and wild-type plants. Their population increases were decreased by 42.7% after three days and 62.2% after five days in the mutant plants compared to the controls (**Table 3**).

4. Discussion

The precise distribution of photosynthetically derived carbohydrate products in the sinks through sucrose transporters is a critical step in completing the life cycle of plants. The fine-tuning of sucrose concentration during various stresses is very important. During the course of evolution, heterotrophs evolved to utilize the benefits of plant photosynthesis and phloem transportation. Viruses move through it and *Cuscuta*, *Orobanche*—like parasitic weeds make a contact with the phloem of the host cell for their nutrition [16]. Herbivorous insects reroute the carbon and nitrogen compounds, such as the grasshopper-induced allocation of carbon to roots [17] and the aphid-induced nitrogen and carbon relocalization in the celery plant that have been reported [18]. Plants have evolved several defense mechanisms to respond to herbivores [3]. Plant transporters play an important role in plant defense. Nicotine, an insect neurotoxin, is synthesized in the roots and then translocated to the leaves by the multidrug and toxic compound extrusion (MATE) transporter in *Nicotiana tabacum*, where it functions to protect the plants [19]. Similarly, glucosinolate is transported to the herbivorous attacking site [20]. Sucrose transporters play a crucial role in the phloem loading and unloading of sucrose as well as in sucrose exchanges between the beneficial symbionts and pathogens [21]. Induction of the sucrose transporter after the grazing of herbivores, followed by suppression in the later stage, has been reported in rice seedlings [22]. Kempema *et al.* (2007) [3] reported silver leaf whitefly (SLWF) nymphs feeding mediated induction of SUC1 in *A. thaliana*. Phloem sap suckers, especially aphids, suck huge quantities of sap and secrete a huge amount of sucrose as honey dew and work as a secondary sink in plants for sucrose. In this study, we tried to establish a relationship between the expression patterns of SUC transporters (SUCs) and aphids. We performed the qRT-PCR analysis of SUCs at 2 h, 24 h, 48 h, 72 h, and 96 h intervals of aphid infestation. The expression pattern of SUCs was the same, that is, first induction up to a level, followed by down regula-

Table 2. Aphid infestation choice tests.

Treatment		Average No. of insects per plant (Mean ± S.E)
24 hours	Control	13.15 ± 2.12^{bc}
	Mutant	**5.12 ± 1.37^{a}**
72 hours	Control	19.50 ± 2.25^{c}
	Mutant	**6.12 ± 1.37^{ab}**

Means in Colum carrying same letter are not different significantly ($p > 0.5$) (means were analyzed by one way ANOVA and compared using Duncan's Multiple Range Test).

Table 3. Percent change in population.

		After 3 days		After 5 days	
Treatment	No of insects released/plant	No of aphids/plant	Reduction over control (%)	No of aphids/plant	Reduction over control (%)
Control plants	4	14.5± 3.35	-	24.12 ± 4.75	-
Mutant plants	4	8.4.0± 2.9	42.0	9.12 ± 3.2	62.20

Percent reduction in population increase (P) =100 × 1 − (Ta × Cb)/(Tb × Ca); (where: P = % reduction over control, Ta = population in mutant after, Ca = population in control after, Tb = population in mutant before; Cb = population in control before).

tion, but the time point and fold inducibility were different. In our results, the expression of SUC6 was earlier (24 h) and was the highest aphid responsive (11-fold) compared to the other SUCs, followed by SUC2, which was induced at 48 h. We were unable to find the expression of SUC8. The lowest expression was seen in SUC5 and SUC9 when compared to other SUCs, probably due to the majority of expressions found in the sink tissue rather than in the selected rosette leaf. The expression of SUCs showed similarities with those found in Genevestigator, that is, SUC2 and SUC1 were the highest in the rosette and cauline leaves, followed by SUC3, SUC4, SUC5, SUC6, and SUC9, with the least expression in SUC8. We also compared the qRT-PCR result with the publicly available data set (GSE5525) [13] of 48 h and 72 h aphid-infested *A. thaliana* microarray. The expressions of SUC1, SUC2, SUC3, SUC4, SUC5, and SUC7 were found only at 72 h, but only SUC1 showed significant induction (more than two-fold). Interestingly, the expression of SUC6 was absent in the microarray experiment, but it was the highest expressive in our observation after 24 h of aphid infestation. Earlier, there were assumptions that after the attack of fungus and wounds, the expression of some SUCs was enhanced to provide energy resources to the damaged cells. The inducibility of SUC3 was earlier correlated to nematode infection and wounds. Here, we are assuming that during the aphids attack, the expression of SUCs was first enhanced and later suppressed. This might have occurred due to the earlier efficient transportation of sucrose to energize the cells and the later acquisition of aphids as secondary sink. To complement our assumption, we selected well-characterized and proven SUC2 mutant in which the plant's sucrose exporting behavior was compromised [23], low sucrose transportation through phloem [6], and its maximum expression in the cauline and rosette leaves (Genevestigators). Results related to aphid infestation choice and reproduction performance test showed that in the mutants, the insects were three times less attracted and their population was 50% - 60% less than in wild-type. The poor reproductive performance and less suitability of aphid to SUC2 mutant correlated with the decrease in the expression of SUCs at a later stage of infestation.

5. Acknowledgements

NKD are grateful to the Council of Scientific and Industrial Research (CSIR) India for providing the research fellowship. This work was supported under Council of Scientific and Industrial Research Net Work Projects (NWP03).

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