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Research Article

Antixenotic resistance of eight *Cucumis* genotypes to melon aphid *Aphis gossypii* (Hemiptera: Aphididae) and some associated plant traits

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Abstract: The melon aphid, *Aphis gossypii* (Glover), is one of the major pests of cucurbits and an efficient vector of plant viruses such as Cucumber Mosaic Virus. Host-plant resistance is one of the management strategies that can be used to control this pest. In this study, choice test was conducted to identify antixenotic resistance against melon aphid in eight *Cucumis* genotypes, namely Hormozgan, Bushehr, Guilan, Girtap, Negeen, Sepehr, Pouya and Armenian cucumber. Choice tests were conducted at 25 ± 1 ºC, 60 ± 5% RH and a photoperiod of 16:8 (L: D) h. After introduction of apterous adult aphids to test arena, the number of aphids on each entry was counted at 2, 4, 6 and 24 hours of release. Total phenolic content, NPK essential elements, leaf thickness and leaf trichome density were also measured to discover any association between these factors and aphid host choice. The most antixenosis effect was observed on 'Bushehr'. Increase in antixenosis correlated with increase in leaf trichomes. Antixenosis can be important mode of resistance by reducing host selection and delaying aphid colonization**.** The identification of antixenotic resistance in several genotypes provides additional options for management of this pest. Moreover, the factors associated with this mode of resistance can be considered in plant breeding programs.

Keywords: Antixenosis, *Aphis gossypii*, *Cucumis*, total phenolic content, trichome

Introduction

Aphis gossypii Glover (Hemiptera: Aphididae), a polyphagous pest (van Emden and Harrington, 2007), is very damaging to cucurbits worldwide (Blackman and Eastop, 2008; van Emden and Harrington, 2007). It causes damage through feeding and transmission of plant viruses such as Cucumber Mosaic Virus (CMV) (van Emden and Harrington, 2007). Due to use of numerous chemical insecticides to control of this pest, it has become resistant to organophosphate (Herron *et al.*, 2001; van Emden and Harrington, 2007), carbamate (O'Brien and Graves, 1992; van Emden and Harrington, 2007) and pyrethroid insecticides in various parts of the world (Sun *et al.*, 1994; van Emden and Harrington, 2007). Consequently, other strategies to manage this pest should be considered. Among such control methods, use of resistant or less-favorable crop cultivars as one of the major components of integrated pest management (IPM) programs can be useful.

In general, there are three categories of resistance to arthropods: antibiosis, antixenosis and tolerance. Often the antibiosis and antixenosis overlap, because distinction between

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them is difficult. Antixenosis alters arthropod feeding or oviposition behavior and makes them select an alternate host plant. Some morphological plant factors such as thickened epidermal layer, waxy layer, trichome density or chemical plant compounds such as allelochemicals or toxic compounds can play a part in antixenosis (Smith, 2005). Several studies have been done on antixenosis mode of resistance to melon aphid (Chen *et al.*, 1997; Coleson and Miller, 2005; Klingler *et al.*, 2001; Storer and van Emden, 1995) and other aphids. According to the related studies, it has been proved that visual stimuli and plant volatiles in host affect aphid's landing. After landing, plant morphology and chemistry can alter aphid behaviour for settling or escaping (van Emden and Harrington, 2007).

In this study we have evaluated antixenosis resistance of eight *Cucumis* genotypes to melon aphid. Furthermore, the probable role of some plant factors including total phenolic content, NPK essential elements, leaf thickness and leaf trichome density associated with this mode of resistance was studied. Finding the resistant genotypes can be useful for keeping the size of aphid population under economically injurious levels. Moreover, understanding the plant characteristics associated with the resistance can be useful in plant breeding programs.

Materials and Methods

Plant materials

In this experiment eight *Cucumis* genotypes including three native cucumbers (Hormozgan, Bushehr and Guilan), four greenhouse cucumber cultivars (Girtap, Negeen, Pouya and Sepehr) and Armenian cucumber (*Cucumis melo* var. *flexuosus*) were tested for antixenosis. Seeds of native genotypes were obtained from Seed and Plant Improvement Institute, Karaj, Iran. The seeds were sown in 20-cm plastic plots filled with fertilized field soil and maintained in the greenhouse condition at 25 ± 1 °C, 60 ± 10% RH and a photoperiod of 16:8 h (L: D).

Aphid colonies

Colonies of *A. gossypii* were initiated by individuals of the aphids collected from cucumber fields in Tehran, Iran. The stock was maintained on potted *Cucumis sativus* var. Beith alpha in screened cages in greenhouse condition at 25 ± 3 °C, 60 ± 10% RH and a photoperiod of 16: 8h (L: D).

Antixenosis

To evaluate antixenosis resistance of these genotypes to melon aphid we used detached leaf choice tests for adult aphids. The tests were conducted within growth chambers in laboratory condition at 25 ± 1 °C, 60 ± 10% RH and a photoperiod of 16: 8 h (L: D).

Choice tests

One detached leaf from fifth or sixth leaf of each genotype was used for this test. The leaves were arranged in a circular arena in a completely randomized design with 10 replicates for each accession of each test. Eighty apterous adult of aphids released on a filter paper (8-cm diameter) were placed at the center of the circle. Dishes were closed using a net to prevent aphids from escaping and placed in a climate room. The number of aphids on each leaf discs was counted after 2, 4, 6 and 24 hours.

Leaf trichome density

To estimate leaf trichome density, we counted the number of trichomes on the abaxial leaf surface in a 1-cm^2 area using a compound microscope (Gonzales *et al.*, 2008).

Leaf thickness

A digital micrometer was used to measure thickness of the leaves, took care to ensure a constant pressure by using the instrument's ratchet clutch and the leaflet mid and lateral ribs were avoided in measurements (White and Montes-R, 2005).

Essential elements (NPK)

The amount of nitrogen (N), potassium (K) and phosphorus (P) were measured according to methods of Kjeldahl (1883), Olsen (1954) and

Jackson (2005), respectively. These tests were done in Research Institute of Forests and Rangelands of Iran.

Total phenolic content

To measure phenolic compounds, 0.2 g of dried leaves was extracted with 10 ml of 80% ethanol. The extracts were centrifuged at 10000 rpm for 20 minutes. The ethanol in the extracts was removed by rotary evaporation. The deposit was dissolved in distilled water. Total phenolic content was determined with Folin-ciocalteu reagent (Sadasivam and Manickam, 1992) using gallic acid as a standard of phenolic compounds. The concentration of total phenol content was measured as milligrams of gallic acid equivalent (mg GAE/g dry extract). The reaction mixture contained 3 ml of ethanol solution of extract, 0.5 mL of Folin-ciocalteu reagent, and 2 mL of 20% (w/v) sodium carbonate that was kept at ambient temperature. After one hour, the absorbance was measured at 650 nm. All treatments were measured in three replicates.

Statistical analysis

After normalization of data, antixenosis effects of the genotypes were tested using ANOVA for the time intervals. Repeated measures analysis was used for assessing the overall antixenosis. The data were grouped by Tukey's test. Pearson correlations were calculated to find out which plant traits have role in antixenosis.

Results

According to the choice test two hours after releasing aphids, the number of aphids on Hormozgan, Bushher, Guilan and Armenian cucumber was lower than on the other genotypes ($F_{7,72} = 7.489$, $P < 0.05$). The most antixenosis effect after 4 hours was recorded for Bushher and Guilan ($F_{7,72} = 30.796$, $P < 0.05$). The number of aphids at third time evaluation ranged from 3.85 aphids on Bushehr to 7.4 aphids on Sepehr ($F_{7,72} = 8.984$, $P < 0.05$). After 24 hours, the least number of aphids settled on Bushher ($F_{7,72} = 10.771$, $P < 0.05$) (Table 1). The greatest differences were detected at 6 h after melon aphid introduction. According to the results of repeated measures design, there was significant difference in number of aphids on the genotypes (Table 2). And the most overall antixenotic effect to melon aphid was observed in 'Bushehr', whereas Sepehr and Negeen exhibited little or no antixenosis (Table 1).

Table 1 Mean (± SE) number of melon aphids on eight *Cucumis* genotypes in several sampling times.

Genotypes	Number of aphids per leaf disc (± SE)				
	2 h	4 h	6 h	24 h	Mean
Hormozgan	4.70 ± 0.517 abc	5.40 ± 0.763 abc	5.30 ± 0.667 bc	4.50 ± 0.453 cd	4.975 ± 0.295 cd
Bushehr	4.30 ± 0.559 c	3.10 ± 0.482 c	3.80 ± 0.533 b	3.40 ± 0.582 d	3.675 ± 0.295 d
Guilan	4.60 ± 0.371 bc	3.70 ± 0.423 bc	5.80 ± 0.573 ab	5.50 ± 0.687 abcd	4.900 ± 0.295 cd
Armenian cucumber	5.20 ± 0.663abc	5.90 ± 0.567 ab	6.20 ± 0.814 b	5.10 ± 0.605 bcd	5.600 ± 0.295 bc
Girtap	6.20 ± 0.663 abc	7.90 ± 0.706 a	5.10 ± 0.482 ab	7.30 ± 0.423 ab	6.625 ± 0.295 ab
Negeen	7.60 ± 0.833 a	7.30 ± 0.616 a	5.20 ± 0.712 ab	8.20 ± 0.712 a	7.075 ± 0.295 a
Pouya	5.70 ± 0.923 abc	6.00 ± 0.471 ab	5.30 ± 0.633 ab	5.10 ± 0.900 bcd	5.525 ± 0.295 bc
Sepehr	7.50 ± 0.428 ab	6.80 ± 0.490 a	7.40 ± 0.236 a	6.30 ± 0.473 abc	7.000 ± 0.295 a

* Means in a column followed by the same letters are not significantly different (Tukey's test at 5% significance level).

Table 2 Repeated measures variance analysis of genotype effects on aphids density in choice test.

Source of variations	SS	df	Mean of square	F	*P*
Genotypes	389.472	7	55.638	16.003	0.01
Error	250.325	72	3.477		

The amount of measured plant factors are summarized and illustrated in Table 3. There was no significant different among the genotypes with respect to leaf thickness ($F_{7,16}$ = 1.504, P = 0.213) and NPK contents ($F_{7,16}$ = 2.352, P = 0.127 for N; $F_{7,16}$ = 1.515, P = 0.286 for P and $F_{7,16}$ = 1.194, P = 0.401 for K) but significant differences in the leaf trichome density and phenolic content were observed. The highest trichome density and total phenolic content were recorded for Armenian cucumber. On the basis of Pearson correlation coefficient, there was a negative correlation between leaf trichome density and number of aphids. But there was no relationship between the number of aphids and leaf thickness, total phenolic content, and NPK (Table 4).

Table 3 Means (± SE) of some measured features of *Cucumis* genotypes.

Genotypes	N (%)	P (%)	K (%)	TPC (ppm)[1]	Thickness (mm)	Trichome density (mm^{-2})
Hormozgan	4.135 ± 0.135	0.495 ±.015	3.850 ± .150	884.815 ± 54.335 a	0.350 ± .027	38.720 ± 1.620 abc
Bushehr	4.085 ± 0.285	0.485 ± .025	3.250 ± .250	794.149 ± 12.331 ab	0.355 ±.031	43.802 ± 1.502 ab
Guilan	4.310 ± 0.600	0.500 ± .040	4.600 ± .600	634.703 ± 29.738 bc	0.375 ± .021	36.205 ± 2.099 bcd
Ar. Cu.	3.740 ± 0.370	0.510 ± .150	5.150 ± .350	995.965 ± 46.085 a	0.430 ± .026	45.160 ± 5.150 a
Girtap	4.290 ± 0.120	0.480 ±.010	3.350 ± .150	426.425 ± 44.297 d	0.405 ± .012	28.200 ± 4.054 d
Negeen	3.555 ± 0.125	0.365 ± .025	3.200 ± .300	373.575 ± 27.222 d	0.357 ± .030	28.450 ± 1.743 d
Pouya	2.970 ± 0.080	0.315 ± .045	4.700 ± .300	513.667 ± 52.208 bc	0.373 ± .024	29.375 ± 2.340 d
Sepehr	3.615 ± 0.285	0.395 ± .035	4.100 ± 1.70	448.608 ± 51.658 bc	0.350 ±.011	30.225 ± 1.924 cd
F (df = 7, 16)	2.352	1.515	1.194	41.965	1.504	12.920
	0.127	0.286	0.401	< 0.05	0.213	< 0.05

Abbreviations: N: nitrogen; P: Phosphor; K: potassium and TPC: total phenol content; Ar. Cu.: Armenian cucumber.
[1] Means in a column followed by the same letters are not significantly different (Tukey's test at 5% significance level).

Table 4 Pearson correlation coefficient (r) between number of aphids *Aphis gossypii* and some plant factors which may have role in antixenosis of Cucumis genotypes to melon aphid.

	N	P	K	TPC	Thickness	Trichome density
Number of aphids	-0.315	-0.417	-0.107	-0.683	0.099	-0.748*

Abbreviations: N: nitrogen; P: Phosphor; K: potassium and TPC: total phenol content.
*: Significant $p < 0.05$.

Discussion

We tested *Cucumis* genotypes for antixenosis to *A. gossypii* by assessing feeding deterrence and aphid settling in choice test. Although winged aphids choose host plants and colonize them in the field (Smith, 2005), we used apterous aphids to detect antixenosis, because their handling is easier than alate ones (Diaz-Montano *et al.*, 2006; Hesler and Dashiell, 2008; Hesler and Dashiell, 2011; Hill *et al.*, 2004). The genotypes with lowest number of aphids on them have the highest antixenosis resistance. Therefore, in our study the most antixenotic effect belonged to Bushehr.

Some genetic attributes cause a plant of one cultivar or species to be less damaged by insects than the susceptible ones which lack these qualities (Kamel and El-Gengaihi, 2009). In antixenosis some morphological or chemical plant factors alter the aphid behaviour, causing the selection of an alternate host plant (Smith, 2005). In this study, antixenosis in the *Cucumis* genotypes was positively correlated with morphological features. At different test times there were aphid density fluctuations on some genotypes. One reason for such fluctuations may be diurnal changes in the phloem sap composition (van Emden and Harrington, 2007; Winter *et al.*, 1992); changes in concentration

of some amino acids and sugars may cause aphids to stop feeding and to pull out their stylets as shown in *Nasonovia ribisnigri* (Mosley) on lettuce (*Lactuca sativa*) and *Aphis fabae* Scopoli on beans (Van Helden *et al.*, 1993). Another reason may be an increase in mobility of individuals in dense colony by tactile disturbance from other members as in colonies of *Drepanosiphum platanoidis* (Schrank) (Dixon, 2012) or attributed to volatile semiochemicals as in colonies of *Rhopalosiphum padi* (L.), (Quiroz *et al.*, 1997). The allelochemicals can be as stimulant or deterrent for the aphids (Smith, 2005).

The antixenosis was positively correlated with leaf trichome density. The role of leaf trichomes is generally water control and resistance against herbivory in some plants (Gonzales *et al.*, 2008). The simple trichomes of these genotypes probably act as mechanical barriers that hinder insect movement and/or feeding (Le Roux *et al.*, 2008;Levin, 1973;Smith, 2005).

There are some works on antixenosis of cucurbits against melon aphid. "Vat" gene has been identified in melon germplasm (Pitrat and Lecoq, 1984) that confers both antibiotic and antixenotic melon resistance to *A. gossypii*. *C. melo* cv Virgos has been identified as resistant cultivar to melon aphid (Martín and Fereres, 2003). JY30 and EP6392 were proved as susceptible and resistant cucumbers to *A. gossypii* (Liang *et al.*, 2015). Lines A and P of melon are known to have high antixenosis resistance (Martín and Fereres, 2003). Higher concentration of both cucurbitacins and phenolic content in globe cucumber in comparison with cucumber plants is reported as the cause of resistance to *A. gossypii* (Kamel and El-Gengaihi, 2009). In some cucurbits more glandular trichomes on leaves of the melon aphid-resistant genotype has been reported (Sarria *et al.*, 2010). Some studies have focused on resistance evaluation of *Cucumis* genotypes to its other major pests (Baldin and Beneduzzi, 2010; Basij *et al.*, 2011; Boissot *et al.*, 2003; Knipping *et al.*, 1975; Mohammadi *et al.*, 2015; Ponti, 1978; Soria *et al.*, 1999) but the genotypes differ in various parts of the world and the commercial verities change by time. Hence, it is hard to introduce a resistant genotype to some major pests. However, it is possible to find some features that contribute to in multi-pest resistance.

Plant acceptance is a critical phase for aphid colonization and population establishment (Le Roux*et al.*, 2008). Antixenosis can deter aphids, reduce colonization and keep the size of population under economically injurious levels (Hesler and Tharp, 2005; Hesler and Dashiell, 2011). Deterrence form settling on host plants may cause aphid to continue searching. Aphids maybe exhausted after long time searching or be preyed before finding a suitable host plant for feeding and reproduction (Hesler and Dashiell, 2011). Aphids initially invade crops in low numbers, then populations increase gradually to reach damaging levels. For these pests, low-to-moderate levels of antixenosis and antibiosis can be effective (Hesler and Tharp, 2005). So, we have focused on evaluation of antixenosis in *Cucumis* against *A. gossypii*. Such findings in combination with information on other resistance mechanisms (Doryanizadeh *et al*, 2016) can be helpful in IPM programs of cucumbers.

Conclusion

The results of this project demonstrated that there are differences between the genotypes, in terms of preference and choice. It was also demonstrated that antixenosis of *Cucumis* correlated positively with leaf trichome density. These characteristics can be considered in breeding programs of *Cucumis*.

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مقاومت آنتی‌زنوزی ژنوتیپ‌های خیار *Cucumis* به شته جالیز *Aphis gossypii* و برخی از ویژگی‌های گیاهی مؤثر در بروز آن

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چکیده: شته جالیز، *Aphis gossypii* (Glover)، یکی از آفات مهم کدوییان و ناقل برخی از ویروس‌های گیاهی از قبیل ویروس موزاییک خیار است. یکی از راه‌کارهایی که می‌تواند در کنترل این آفت به‌کار رود، استفاده از گیاه میزبان مقاوم است. در این پژوهش از آزمون انتخابی برای ارزیابی مقاومت آنتی‌زنوزی هشت ژنوتیپ خیار، با نام‌های ژنوتیپ‌های هرمزگان، بوشهر، گیلان، گیرتاپ، نگین، سپهر، پویا و خیار چنبر، به شته‌ی جالیز استفاده شده است. آزمون انتخابی در دمای ۱ ± ۲۵ درجه‌ی سلسیوس، رطوبت نسبی ۵ ± ۶۰ درصد و دوره‌ی نوری ۱۶: ۸ ساعت (روشنایی: تاریکی) انجام شد. پس از رهاسازی شته‌های بالغ بی‌بال، تعداد شته‌ها روی هر ژنوتیپ در بازه‌های زمانی ۲، ۴ و ۶ ساعت پس از رهاسازی شمارش شدند. برای یافتن ارتباط بین تعداد شته روی هر ژنوتیپ با ویژگی‌های گیاه، مقادیر ترکیبات فنلی کل، عناصر ضروری NPK، ضخامت برگ و تراکم تریکوم‌های برگ نیز اندازه‌گیری شد. براساس نتایج حاصل، بیش‌ترین مقاومت آنتی‌زنوزی روی ژنوتیپ بوشهر مشاهده شد. افزایش مقاومت آنتی‌زنوزی با افزایش تریکوم‌های برگ هم‌بستگی داشت. آنتی‌زنوز یکی از مکانیسم‌های مهم مقاومت تلقی می‌شود که می‌تواند انتخاب میزبان را کاهش می‌دهد و تجمع شته روی گیاه را به تأخیر اندازد. تشخیص مقاومت آنتی‌زنوزی در ژنوتیپ‌های خیار گزینه‌های بیش‌تری برای مدیریت این آفت در اختیار ما قرار می‌دهد. هم‌چنین فاکتورهای مؤثر در بروز این مقاومت می‌توانند در برنامه‌های به‌نژادی مورد استفاده قرار گیرد.

واژگان کلیدی: آنتی‌زنوز، *Cucumis*، *Aphis gossypii*، ترکیبات فنلی کل، تریکوم