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Research Article

Investigating the physiological and morphological responses of *Cucumis sativus* to *Phelipanche aegyptiaca* parasitism

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Abstract: A greenhouse experiment was conducted to examine the influence of *Phelipanche aegyptiaca* on vegetative growth, rate of photosynthesis, chlorophyll fluorescence and leaf chlorophyll content of 35 cucumber genotypes. High demand of assimilates by *P. aegyptiaca* caused significant reductions in shoot and root dry weight, leaf number, leaf area and plant height in all cucumber genotypes. Once plants were infected by *P. aegyptiaca*, the leaf chlorophyll content, the photosynthesis rate and the maximum quantum yield of *PSII* chemistry were significantly less than control, thus implying a reduction in carbon assimilation, photosynthesis efficiency and susceptibility of infected plants to photoinhibition. *P. aegyptiaca* traits were significantly affected by cucumber genotypes. There was no correlation between *P. aegyptiaca* traits with the reduction percentage of cucumber shoot dry weight. However, there were correlations between underground attachments number plant^{-1} (UAN) and percentage of cucumber root dry weight reduction (-0.58), total attachment number plant $^{-1}$ (TAN) and the percentage of reduction of root dry weight (+0.39). In accordance with the results obtained, the genotypes were classified into 3 groups. It was demonstrated that the genotype number 22 (Khassib) behaved differently to other genotypes and, in particular, they suffered less damage from the presence of *P. aegyptiaca*.

Keywords: Chlorophyll content, Chlorophyll fluorescence, Parasitic plant, Photosynthesis rate

Introduction

One of the most important members of the Cucurbitaceae family is the *Cucumis sativus*. It is an economically important crop cultivated worldwide, occupying around 77829 ha in Iran and producing approximately 1,981,130 tonnes of fruit (FAO, 2017). This amount of production requires careful investigation of yield reducing factors. Parasitic plants are one of the important factors that reduce the amount of production, and there are still no effective means available to deal with them.

P. aegyptiaca is a chlorophyll-lacking obligate holoparasite of dicotyledonous species. It can damage many plant families, including Solanaceae, Fabaceae, Apiaceae, Asteraceae, and Cucurbitaceae (Eizenberg *et al.*, 2004; Irving and Cameron, 2009; Parker, 2009; Gevezova *et al.*,

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2012; Joel *et al.*, 2013. Crop losses due to *P. aegyptiaca* can vary from 5-100% (Buschmann *et al.*, 2005; Hershenhorn *et al.*, 2009; Motazedi *et al.*, 2010). The potential damage that *P. aegyptiaca* can cause in crops is influenced by various biotic and abiotic factors like the temperature (Ephrath *et al.*, 2012), crop sowing date (Rubiales *et al.*, 2003; Grenz *et al.*, 2005), soil organic matter content (Heidar and Sidahmed, 2003; Mahgoub *et al.*, 2012), nutrition management (Labrousse *et al.*, 2010), irrigation (Parker and Riches, 1993) and host factors including plant genotype (Pérez-de-Luque *et al.*, 2005).

Several methods have been proposed for *P. aegyptiaca* control in the field, such as chemical control, soil solarization, arbuscular mycorrhizal fungi symbiosis, etc. (Goldwasser and Kleifeld, 2004; Eizenberg *et al.*, 2012; Hosseini-Faradonbeh *et al.*, 2021). However, none of these methods have been able to reduce *P. aegyptiaca* damage sufficiently. This has led to a search for genotypes resistant to *P. aegyptiaca* (Zahar *et al.*, 2003; Buschmann *et al.*, 2005; Fernandez-Martınez *et al.*, 2008; Scholes and Press, 2008; Hosseini-Faradonbeh *et al.*, 2020) as it has been found in other Orobanche species. For example, Bardaro *et al.* (2016) proved that pea resistance to *Orobanche crenata* is due to a lower exudation of strigolactones. Similarly, Qasem and Kasrawi (1994) found a high to moderate level of resistance between tomato cultivars and wild accessions to *Orobanche ramose*. In legumes, only moderate to low levels of resistance against *O. crenata* have been reported (Rubiales *et al.*, 2006; Pérez-de-Luque *et al.*, 2009; Sillero *et al.*, 2010). In chickpea, necrosis of host cell tissue in contact with *O. crenata* was reported by Rubiales *et al.* (2003). According to the literature cited, the best long-term strategy to control P. aegyptiaca could be through identifying and breeding resistant crop genotypes.

Based on the farmer's oral reports and the author's observations, *P. aegyptiaca* can damage cucumber production in Iran farmlands and greenhouses, and there is no efficient control method to prevent yield losses. To overcome t this problem, the first step is the identification of cucumber cultivars with differentiated physiological and morphological responses to infestation. Therefore, the objective of this study was to investigate the influence of *P. aegyptiaca* on the vegetative growth, rate of photosynthesis, chlorophyll fluorescence and leaf chlorophyll content of 35 cucumber genotypes. This could help farmers choose cultivars most resistant to *P. aegiptiaca.*

Materials and Methods

The experiment was conducted at Isfahan University of Technology, Iran from May to July 2017. The greenhouse has a transparent PVC cover, and the mean daily greenhouse temperature ranged from 25/15 ºC, and the relative humidity was set at 65-75%. Thirty-five genotypes, including 17 domestic (non-commercial), eight commercial greenhouse-grown, and ten commercial field genotypes commonly cultivated in Iran, were studied (Table 1). The experiment was carried out using a completely randomized design with six replications.

Table 1 Cucumber genotypes characteristics and given number to each genotype used in the experiment.

Domestic genotypes			Greenhouse-grown genotypes			Field genotypes			
Genotype	No.	Origin	Genotype	No.	Origin	Genotype	No.	Genotype	No.
55950	1	Kurdistan	56013	11	Tehran	Storm	18	Baran	26
55952	2	Fars	56032	12	Gillan	Negin	19	Superdomino	27
55956	3	Yazd	56043	13	zanjan	Keyhan	20	Omid	28
55957	4	Markazi	56044	14	Zanjan	Alfarid	21	Emprator	29
55960	5	Yazd	56046	15	Khorassan	Khassib	22	Clause	30
55961	6	Azarbaijan	Dastgerd	16	Naein	Spadana	23	Bingo	31
55963	7	Hamadan	Kharvan	17	Isfahan	Newsun	24	Grifaton	32
55995	8	Mazandaran				Kaspian	25	Kaveh	33
56002	9	Azarbaijan						Pop	34
56005	10	Booshehr						Argeto	35

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Twelve pots were considered for each genotype. Six pots were sown with each cucumber genotype without *P. aegyptiaca* seed contamination as control treatments and the rest of the pots were sown with *P. aegyptiaca* seeds as a contaminated treatment. The *P. aegyptiaca* seeds were collected from one infected tomato farmland (to minimize the effect of environmental conditions on broomrape seeds). To break dormancy and improve *P. aegyptiaca* seed germination, the seeds were soaked in 30 mg L^{-1} gibberellic acid solution for 1 week at 18 ºC and incubated in darkness (Teimouri *et al.*, 2016). Three cucumber seeds were sown per pot (30 cm in height and 25 cm in diameter) and thinned to one plant per pot after plant establishment.

In order to facilitate the measurement of the traits, a soilless substrate (fine perlite 50%, sand 50%) was used to fill the pots. After filling two thirds of the pots in the infected treatments, 50 mg kg^{-1} of *P. aegyptiaca* seeds were mixed with the bed (El-Halmouch *et al.*, 2006) and then the cucumber seeds were planted. At the two-leaf stage of the cucumber seedlings, a fungicide (Mancozeb M45 WP80%) was used to prevent seedling damping-off. Irrigation was carried out according to the needs of the plant and to field capacity; the pots were fed with a Hoagland diet (Hoagland and Arnons, 1983) according to a common nutritional plan.

Data collection

Different traits were measured on cucumber genotypes and *P. aegyptiaca* plants.

Assessments during the growing season

Cucumber plant assessments were made during the growing season, after the emergence of at least one *P. aegyptiaca* stem in all treated pots, based on the desired assessment average in the third fully developted leaves in the last two-thirds of each plant.

Net photosynthesis rate (PN) was measured with the calibrated portable gas-exchange system (*LCi, ADC Bioscientific Ltd.,* UK) from between 08:00 to 11:00 h when temperature ranged between 21 and 25 ºC and photon flux density was 1250–1700 µmol m^{-2} s^{-1} in the dark adapting the young fully-expanded leaves for 20 minutes. The maximum quantum yield of PSII (fv/f_m) was measured using a portable chlorophyll fluorometer (*Opti-Sciences, Inc., Hudson,* NH, USA). To gauge the content of leaf chlorophyll *a*, 0.3 gr of fully-expanded healthy leaves were ground as a sample. The extract was purified with 10 mL of 80% (v/v) acetone (Lichtenthaler and Wellburn, 1983), and the absorbance was measured at 646.8 and 663.2 nm to quantify Chlorophyll *a* by a UV-visible spectrophotometer (*HITACHI, U 1800,* Japan) according to equation 1.

$$\mathrm{Chl}a(\mathrm{mg/ml}) = 12.25A663.2 - 2.79A646.8 \quad (1)$$

Where Chl*a* is the content of chlorophyll a, and A is the absorbance in mentioned wavelength, respectively

Assessments at the end of the growing season

Other traits were measured 90 days after planting (end of the experiment) including cucumber plant height, leaf number, and leaf area (by using leaf area measurement device model WIN AREA-UT-11 and the means of 3 adult leaves per each treatment), and shoot and root dry weight (by drying the fresh cucumber shoot and root at 60 ºC for three days). In infected pots, additional traits were assessed including underground attachments number $plant^{-1}$ (UAN), emerged spikes number $plant^{1}$ (ESN), total attachment number $plant^{-1}$ (TAN), and attachment dry weight (g) $plant^{-1}$ (ADW). These traits were counted after sieving the soil of the infected pots and washing the cucumber roots. To calculate the amount of ADW, a whole attachment was dried at 60 ºC for three days and then weighed.

Statistical analysis

For every trait, the percentage of change in infected genotypes compared to the control was calculated (Mauromicale *et al.*, 2008) according to the following formula:

$$Change\ (\%) = [(b-a)/a] \times 100 \quad (2)$$

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Where ‘a’ is the mean value of the trait in non−infected plants, and ‘b’ is the mean value of the trait in infected plants.

Before analysis, the normality of data was checked (Shapiro-Wilk test), which showed that no statistical data transformation was necessary. Mean values for uninfected plants for each trait were also presented. Generalized linear models employed in PROC GLIMMIX of SAS (version 9.4; SAS Institute, Gary, NC) were used to analyze the effect of treatments on response variables. The least squared means (LSMEANS) statement of GLIMMIX procedure in SAS was used to compare treatment means at 5% significance level according to Fisher's Least Significant Difference (Fisher's LSD). Pearson’s correlation coefficients were calculated to assess the relationships between *P. aegyptiaca* traits and the reduction percentage of cucumber shoot and root dry weight.

To classify cucumber genotypes according to all traits related to cucumber and *P. aegyptiaca*, multivariate statistical analysis and classification methods were employed using cluster analysis. For this purpose, the matrix of similarity was calculated, and by the use of between-group linkage and squared Euclidean distance measurements, a dendrogram was drawn for cucumber genotypes.

Results

Cucumber traits

The results of the analysis of variance and mean comparison of all traits are summarized in Tables 2, 3, and 4. The investigation of the changes of leaf area indicated that contamination with *P. aegyptiaca* caused a significant decrease in leaf area of the infected cucumber genotypes as compared to the control. According to the results, *P. aegyptiaca* in different genotypes caused a decrease in cucumber leaf area ranging from 17.86 and 80.42 %. Mean comparison of data showed that the lowest percentage of the leaf area reduction was related to the cultivar 17, which showed no significant difference with leaf area reduction in genotypes 8, 6, 30, 5, and 9. The highest percentage of leaf area reduction was observed in the native genotype 12 (80.14%), but with no statistically significant difference to genotypes 11, 24, 2, 13, 25, 34, 33, 32, 15, 27, 3, 19, 7, 14, 23 and 4 (Table 3).

Table 2 Analysis of variance for change percentage of cucumber traits.

Source of variation	Means of square		
	Residual	Genotype	Total
Leaf area	349.00	1835.13**	590.76
Leaf number	94.97	1141.78**	265.27
Height	47.18	894.39**	185.00
Shoot dry weight	29.34	313.11**	75.50
Root dry weight	233.76	721.97**	314.15
Chlorophyll *a*	19.22	826.06**	216.57
Photosynthesis rate	61.65	1089.92**	228.93
Fv/Fm	56.15	298.16**	95.52
Degree of freedom (df)	175	34	209

In the presence of *P. aegyptiaca*, cucumber leaves decreased significantly. Results showed that the least damage occurred in genotypes 16, 28, and 14, with 17.90, 20.92, and 24.04% reduction compared to their controls, respectively. While genitypes 29 (73.17%), 32 (71.05%), 8 (69.84%), 30 (66.43%), 18 (65.57%), 24 (63.57%), 19 (62.69%) and 7 (62.08%) were the most affected.

In response to *P.aegyptiaca*, the height of cucumber genotypes was significantly reduced. Height reductions were greatest in genotypes 18 (90.59%), 32 (84.65%) and 24 (84.13%). In contrast, the least damage was observed in 16 and 28, with a 37.64 and 38.14% decrease relative to their controls, respectively. It is clear that changes in leaf area, leaf number, and plant height affect cucumber shoot dry weight. Shoot dry weight decreased severely from 51 to 91% in all the infected genotypes. The lowest and the highest dry weight loss of the shoot were observed in the greenhouse genotype 22 (51%) and native genotype 2 (91%), respectively. Cucumber root dry weight was significantly affected by *P. aegyptiaca* in different genotypes. The least damage to root dry weight was seen in 17 (46.53%) and 25 (59.53%) genotypes. Additionally, the decrease in root weight compared to their controls was more than 95% in genotypes 3, 5, 8, 11, 1, 13, 34, 22, and 21.

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Table 3 Effect of infection with *Phelipanche aegyptiaca* on leaf area, leaf number, height and shoot dry weight of cucumber genotypes.

Genotype No.	Leaf area		Leaf number		Height		Shoot dry weight	
	Non-infected plants (mm^2)	Change (%)	Non-infected plants	Change (%)	Non-infected plants (cm)	Change (%)	Non-infected plants (g)	Change (%)
1	19889.05*	51.04	19.17	33.12	127.00	63.54	16.85	86.88
2	15515.81	74.65	24.17	62.03	141.50	74.06	18.57	91.65
3	13435.62	65.47	21.33	53.28	129.67	65.23	18.32	84.14
4	15048.28	60.87	21.83	55.35	122.67	59.16	18.05	88.70
5	11068.79	30.22	18.50	47.52	123.50	62.05	17.39	77.57
6	16205.5	25.28	20.83	51.41	120.33	66.31	18.30	81.32
7	13728.01	64.50	27.67	62.08	185.17	67.74	15.16	85.39
8	13257.21	22.66	24.33	69.84	160.58	75.63	17.95	87.47
9	13117.55	30.56	20.67	61.16	104.00	69.13	18.22	87.72
10	12299.36	51.88	21.17	60.73	190.08	80.91	16.74	82.87
11	26111.21	79.34	18.83	57.87	203.08	77.96	16.80	87.15
12	25883.1	80.42	22.83	51.90	179.75	56.99	17.18	89.10
13	23737.4	74.49	20.50	58.29	142.42	72.03	13.00	83.31
14	13638.77	63.17	20.33	24.04	142.25	52.39	12.12	85.41
15	13749.72	67.80	26.33	57.27	177.33	69.73	14.12	87.72
16	12629.14	39.69	19.50	17.90	104.42	37.64	14.47	73.45
17	14040.77	17.86	22.00	43.69	154.33	64.77	14.23	86.61
18	18328.3	51.24	29.50	65.57	228.50	90.59	14.50	80.79
19	17312.11	64.76	21.17	62.69	124.92	76.01	13.30	90.15
20	18444.79	53.20	25.33	59.91	160.58	81.85	14.00	82.08
21	19240.51	46.97	22.50	50.80	156.50	71.25	16.40	75.33
22	19366	44.71	19.50	42.27	154.67	76.19	17.88	55.67
23	15664.22	62.49	19.33	34.37	178.58	57.51	13.02	74.57
24	19191.08	78.26	23.50	63.57	204.50	84.13	16.84	86.57
25	20625.87	72.78	18.00	48.03	162.58	80.35	15.13	76.23
26	15665.52	46.99	29.17	56.30	198.58	80.42	13.91	82.86
27	16114.53	67.60	20.00	58.86	116.76	66.06	12.10	77.16
28	16371.1	44.11	21.17	20.92	140.00	38.14	16.40	77.58
29	17041.03	58.97	32.17	73.17	143.00	82.36	10.85	90.53
30	13377.48	25.91	30.50	66.43	158.92	68.61	11.50	80.99
31	14715.63	56.02	25.00	59.71	165.17	77.66	12.70	82.82
32	17692.24	69.23	27.67	71.05	188.58	84.65	12.30	87.72
33	22855.63	69.51	23.33	47.91	150.00	77.48	13.00	73.02
34	13444.61	72.17	17.83	55.03	140.00	78.60	15.02	87.40
35	15847.35	48.40	19.33	36.45	137.75	55.26	14.67	70.93
LSD (5%)		21.28		11.10		7.8		6.17
CV (%)		33.82		18.53		9.84		6.58

Values are means of 6 measurement dates.
In each trait percentage of changes in infected plants related to non-infected plants.
**,significantly different at $P \leq 0.01$.

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Table 4 Effect of infection with Phelipanche aegyptiaca on Root dry weight, Photosynthesis rate, Chlorophyll a (Chl a) and maximum quantum yield of PSII chemistry (Fv/Fm) of cucumber genotypes.

Genotype No.	Root dry weight		Photosynthesis rate		Chl *a*		F_v/F_m	
	Non-infected plants (g)	Change (%)[2]	Non-infected plants (µmol CO_2 m^{-2} s^{-1})	Change (%)	Non-infected plants (µg ml^{-1})	Change (%)	Non-infected plants	Change (%)
1	7.20	95.91	11.28	36.42	21.74	21.46	0.828	1.17
2	4.98	92.19	13.50	46.05	16.48	40.37	0.820	6.70
3	4.27	95.14	16.35	45.80	14.98	40.62	0.828	9.87
4	2.21	94.42	16.41	51.44	14.43	62.01	0.829	11.79
5	3.45	95.15	16.28	49.74	13.46	61.86	0.825	14.43
6	5.53	89.32	16.25	38.62	28.39	44.66	0.830	29.05
7	5.01	82.05	12.48	42.72	16.64	39.72	0.818	18.48
8	4.92	95.27	11.80	34.29	10.56	35.05	0.813	8.10
9	3.92	94.31	11.55	21.57	20.00	62.21	0.797	13.49
10	4.81	92.86	11.95	33.65	24.31	70.89	0.807	14.28
11	5.98	95.50	12.30	18.79	15.64	17.55	0.812	4.58
12	1.86	80.66	13.75	37.94	16.14	57.50	0.817	11.40
13	5.66	96.37	12.31	42.77	21.45	48.63	0.792	0.75
14	5.01	91.11	228.97	55.05	16.66	43.42	0.820	10.51
15	6.51	92.77	12.88	39.78	17.50	61.36l	0.824	5.88
16	4.15	92.82	10.01	12.56	16.51	42.33	0.814	4.82
17	4.07	46.53	11.27	16.73	13.41	32.28	0.823	8.51
18	4.90	90.03	11.78	36.58	12.86	37.73	0.810	6.75
19	4.91	90.66	13.72	65.38	20.02	41.41	0.816	14.62
20	4.90	91.86	11.47	54.55	24.40	44.82	0.818	30.26
21	5.66	98.01	10.19	20.82	20.58	17.56	0.807	19.65
22	6.81	97.63	9.34	32.22	21.01	44.91	0.792	3.79
23	4.77	93.95	11.93	24.54	23.08	45.06	0.797	3.00
24	3.14	93.29	10.15	20.42	19.39	35.63	0.785	16.27
25	3.48	59.53	13.35	36.79	19.71	29.09	0.808	9.55
26	9.48	93.45	14.64	42.85	17.00	54.30	0.813	0.82
27	6.45	86.80	12.74	64.39	13.87	37.97	0.814	3.61
28	5.74	78.40	12.71	47.99	18.80	60.31	0.814	9.62
29	5.84	83.20	13.32	45.69	11.82	57.47	0.768	6.92
30	5.56	87.55	15.29	57.93	15.60	65.92	0.812	5.24
31	3.88	90.60	15.20	47.18	24.39	66.48	0.807	0.34
32	3.40	90.22	13.59	45.48	21.53	42.06	0.815	7.24
33	2.46	66.42	13.10	48.86	17.13	18.27	0.794	5.12
34	6.18	96.63	12.04	53.38	17.60	48.15	0.816	11.61
35	3.41	85.16	12.58	26.79	14.78	35.89	0.794	11.26
LSD (5%)		17.42		6.14		8.94		8.53
CV (%)		17.28		9.80		19.69		77.25

Values are means of 6 measurement dates.
In each trait percentage of changes in infected plants related to non-infected plants.

The percentage of photosynthesis rate changes in the infected genotypes varied significantly. The highest percentage of photosynthesis reduction was observed in genotypes 19 (65.38%), 27 (64.39%) and 30 (57.93%), and the lowest in 16 (12.56%), 17 (16.73%), 11 (15.64%), 24 (20.42%), and 21 (20.82%). The percentage of photosynthesis decrease in other genotypes varied between 20 and 50%. In all cases, chlorophyll content decreased in the infected treatments compared to the control. The highest percentage of the decrease occurred in genotypes 30 (65.92%), 31 (66.48%), and 10 (70.89%), and the least damage was related to 11, 13, 33, and 1.

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There was also a significant difference in the percentage reduction of the maximum quantum yield of PSII chemistry in infected cucumber genotypes.. The reduction percentage in genotypes 3, 13, 26, 1, 23, 27, 22, 11, 16, 5, 30 and 15 varied from 0.33 to 8.50% (the least damage). The highest percentage decrease was observed in 20 (30.26%) and 6 (29.05%) genotypes. In other genotypes, the percentage of decrease in the trait varied between 9.24 to 14.28%.

Broomrape traits

Data analysis of *P. aegyptiaca* traits showed that different cucumber genotypes affect *P. aegyptiaca* and that the host-parasite has a reciprocal interaction. The difference in the traits measured in the infection treatment was significant between the 35 cucumber genotypes (Table 5 and 6). The results showed that the lowest mean of emerged spikes number plant^{-1} (ESN) (5.33 stems per cucumber plant in each pot) was found in the genotypes 6. This was not significantly different to genotypes 16, 12, 30, 2, 28, 9, 31, 27, 1, 17, 14, 10, 33 and 26.

The highest emerged spike number plant^{-1} (ESN) was observed in genotypes 8 (16.5stems per cucumber plant in each pot), 19, and 15 (13.66 stems per cucumber plant in each pot). In the rest of the genotypes, the average ESN varied between 16.8 to 10.66 stems per cucumber plant with no significant statistical difference calculated (Table 5).

The highest (16.33) and the lowest (0.33) number of underground attachments number plant^{-1} (UAN) was found in genotypes 33 and 16, respectively.

The cucumber genotypes differed in total *P. aegyptiaca* attachment number plant^{-1}(TAN). Genotypes 33 (24.61) and 16 (6.5) had the highest and the lowest total attachment number plant^{-1} (TAN), respectively. Total attachment dry weight (g)/plant $^{-1}$ (ADW) varied from 0.63 to 2.18 grams in cucumber genotypes. The lowest dry weight (0.63 g/plant) was related to the genotypes 2, and the highest to genotype 30 (2.18 g/plant).

No significant correlation between *P. aegyptiaca* traits and the reduction percentage of root and shoot dry weight in cucumber genotypes was demonstrated in this experiment (Table 6). However, there was a negative correlation between the change percentage in cucumber root dry weight and UNA and TAN ($p \leq 0.001$) (Table 7).

Table 5 Analysis of variance for *Phelipanche aegyptiaca*.

Source of variation	Means of squares		
	Residual	Treatment	Total
Underground attachments number plant^{-1} (UAN)	5.90	59.52**	14.63
Emerged spikes number plant^{-1} (ESN)	6.07	30.83**	10.10
Total attachment numbe plant^{-1} (TAN)	11.76	89.54**	524.41
Attachment dry weight plant^{-1} (ADW)	0.14	0.63**	0.22
Degree of freedom (df)	175	34	209

** significantly different at $P \leq 0.01$.

Table 6 Mean of *Phelipanche aegyptiaca* grown with 35 cucumber genotypes.

Genotype No.	Underground attachments number plant^{-1} (UAN)	Emerged spikes number plant^{-1} (ESN)	Total attachment number plant^{-1} (TAN)	Attachment dry weight (g) plant^{-1} (ADW)
1	4.00	7.67	11.67	1.03
2	4.67	6.67	11.33	0.63
3	5.50	8.50	14.00	0.93
4	3.00	8.33	11.33	0.93
5	3.67	10.50	14.17	0.96
6	6.00	5.33	11.33	0.91
7	6.00	10.67	16.67	1.32
8	4.33	16.50	20.83	1.76
9	3.33	6.83	10.17	0.72
10	8.17	7.83	16.00	1.40

Continued in the next page.

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Table 6 continued

Genotype No.	Underground attachments number plant^{-1} (UAN)	Emerged spikes number plant^{-1} (ESN)	Total attachment number plant^{-1} (TAN)	Attachment dry weight (g) plant^{-1} (ADW)
11	10.50	8.50	19.00	1.43
12	7.00	6.00	13.00	0.81
13	6.33	8.17	14.50	1.33
14	7.83	7.83	15.67	1.08
15	9.33	13.67	23.00	1.11
16	0.67	5.83	6.50	0.92
17	13.67	7.67	21.33	1.47
18	5.50	10.33	15.83	1.19
19	5.67	13.67	19.33	1.03
20	6.17	8.17	14.33	0.81
21	3.67	8.83	12.50	1.12
22	6.17	9.00	15.17	1.17
23	8.50	8.50	17.00	1.10
24	7.17	8.67	15.83	1.28
25	8.83	8.83	17.67	0.90
26	4.83	7.83	12.67	1.63
27	8.50	7.50	16.00	1.46
28	5.50	6.83	12.33	1.09
29	9.33	9.83	19.17	1.11
30	3.83	6.50	10.33	2.18
31	12.33	7.00	19.33	1.52
32	8.17	8.67	16.83	1.53
33	16.33	7.83	24.17	1.05
34	7.83	8.17	16.00	1.58
35	8.50	9.17	17.67	1.51
LSD (%)	2.76	2.80	3.90	0.43
CV (%)	35.32	28.57	22.11	31.44

Values are means of 6 measurement dates.

Table 7 Correlation coefficient among *Phelipanche aegyptiaca* traits and reduction percentage of shoot and root dry weight of cucumber genotypes.

	Attachment dry weight (g) plant^{-1} (ADW)	Total attachment number plant^{-1} (TAN)	Emerged spikes number plant^{-1} (ESN)	Underground attachments number plant^{-1} (UAN)
Reduction of shoot dry weight (%)	-0.031 $^{n.s}$	0.066 $^{n.s}$	0.15 $^{n.s}$	-0.026 $^{n.s}$
Reduction of root dry weight (%)	-0.0048 $^{n.s}$	0.39 *	0.13 $^{n.s}$	-0.58**

*,** and n.s indicates correlation at the significance level of 0.05 and 0.01, and the lack of correlation between the desired traits.

It appears that, by increasing the root volume, thechances of root contact with *P. aegyptiaca* seeds in the potting soil were incresed. However, not all nodules are necessarily capable of infecting or causing necrosis, so the percentage loss of cucumber root dry weight was lower than that of the control. The total attachment number plant^{-1}(TAN) was positively and significantly correlated ($p \leq 0.05$) with the percentage change of cucumber root dry weight. Thus, by increasing the total number of *P. aegyptiaca* connections, the reduction percentage of root dry weight decreases, and the plant will be more damaged.

Cluster analysis

The cluster analysis, based on all traits measured in cucumber genotypes and *P. aegyptiaca*,

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allows classification of the cucumber genotypes into three main groups: Cluster 1: includes genotype 22; Cluster 2: genotypes 25, 27, 5, 28, 35, 16, 33, 21 and 23; and Cluster 3: genotypes 3, 7, 14, 30, 6, 18, 20, 13, 10, 26, 31, 2, 29, 19, 4, 12, 17, 24, 1, 11, 32, 8, 34, 9 and 15 (Fig. 1).

Comparison of trait means in percentage decrease in different clusters is summarized in Table 8. In cluster 1, the leaf area change percentage was the lowest, and genotypes 22 compensated for the drastic reduction of root dry weight through less damage to *fv/fm* and photosynthesis rate. In this cluster, the reduction percentage of dry shoot weight, UAN, and TAN were less than in the other clusters. In cluster 2, the damage to the root, height, leaf number, chlorophyll *a*, and also ESN and ADW was less than in the other clusters.

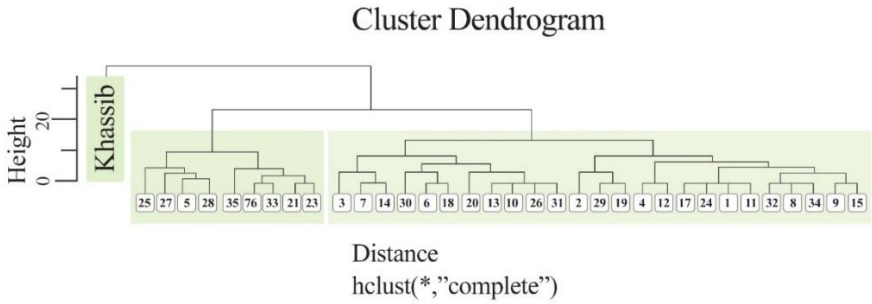

Figure 1 Dendrogram of cluster analysis based on studied traits in cucumber using between-groups linkage. Left to right: cluster 1: included just genotype 22 (Khassib),Cluster 2: included genotypes 25 (Kaspian), 27 (Superdomino), 5 (55960), 28 (Omid), 35 (Argeto), 16 (Dastgerd), 33 (Kaveh), 21 (Alfarid) and 23 (Spadana) and Cluster 3: genotypes 3 (55956), 7 (55963), 14 (56044), 30 (Clause), 6 (55961), 18 (Storm), 20 (Keyhan), 13 (56043), 10 (56005), 26 (Baran), 31 (Bingo), 2 (55952), 29 (Emperator), 19 (Negin), 4 (55957), 12 (56032), 17 (Kharvan), 24 (Newsun), 1 (55950), 11 (56013), 32 (Grifaton), 8 (55995), 34 (Pop), 9 (56002) and 15 (56046) respectively.

Table 8 Means of traits related to 35 cucumber genotypes (%Change) and *Phelipanche aegyptiaca* in different clusters.

Trait	Cluster1	Cluster2	Cluster3
Shoot dry weight	55.67	75.09	85.89
Root dry weight	97.63	84.02	89.67
Height	76.18	60.63	72.81
Leaf area	44.70	53.53	56.26
Leaf number	42.26	40.30	57.41
Chlorophyll *a*	44.91	38.70	46.86
Maximum quantum yield of PSII chemistry (*Fv/Fm*)	3.79	9.00	10.18
Photosynthesis rate	32.22	36.94	41.24
Underground attachments number plant^{-1} (UAN)	6.16	7.12	6.82
Emerged spikes number plant^{-1} (ESN)	9.00	8.20	8.76
Total attachment number plant^{-1} (TAN)	15.16	15.33	15.58
Attachment dry weight plant^{-1} (ADW)	1.17	1.12	1.23

Discussion

It appears that *P. aegyptiaca* represents an additional sink for the host plant to assimilates and, through damage to the photosynthesis capacity of the host plant, reduces the biomass of the shoot and root. However, because the parasitic plant is not a large or significant reservoir of carbon, in most cases, the total amount of parasite and host plant biomass is significantly lower than the non-contaminated host biomass (Barker *et al.*, 1996; Dale and Press, 1998).

Mauromicale *et al.* (2008) also reported that the level of *P. aegyptiaca* damage to photosynthetic indices, including photosynthesis rate and maximum quantum yield of PSII, in tomato genotypes was different. They believe that damage to the quantum function is due to the effect on the *fv* index, which implies damage to

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the electron transfer of PSII. Moreover, other chlorophyll fluorescence parameters, including f0, fm, are significantly reduced in the infected host plants compared to the control.

Other experiments showed that most damage caused by *P. aegyptiaca* to the host is due to reduced carbon assimilation, reduction of photosynthesis, and damage to the photosynthesis system (Khamis *et al.*, 1990; Lima *et al.*, 1999; Demirbaş and Acar, 2017). The damage to the photosynthesis system of the host plant may result from a reduction of chlorophyll content, inhibition of the initial photoreactions, and reduction of the association with the rhizosphere.

In the Musselman (1980) experiment, although the infected plants were more susceptible to photoinhibition, there was no relationship between the degree of damage and the number and biomass of *P. aegyptiaca* in each pot. This is due to the parasite's effect on the balance of host growth hormones by means of the secretion of toxins, and the function of the latter is independent of the number of parasite plants. However, genotypes with higher photosynthesis rates and chlorophyll content are more likely to be less susceptible to photo-inhibition during parasite contamination.

Given that most damage by *P. aegyptiaca* occurs during parasite life stages underground, how the host plant responds to parasitism is very important in determining the final damage and the effectiveness of the control methods. According to the severity of response, *P. aegyptiaca* hosts can be classified as resistant, tolerant, or susceptible. This may be used to identify the source of resistance in plant cultivars. In our study, despite severe infections, there was high genetic variability in response to *P. aegyptiaca* amongst the cucumber genotypes. These results are in accordance with those of other researchers (Certainly, more experiments are needed to reach a definitive conclusion).

Eizenberg *et al.* (2003) showed different clover responses to broomrape. Goldwasser and Kleifeld (2002) reported different responses in parsley as a broomrape host. In other crops like sunflower (Höniges 2008), common vetch (Goldwasser *et al.*, 1999), legumes (Pérez-de-Luque *et al.*, 2010), rapeseed (Buschmann *et al.*, 2005), turnip and carrot (Zahhar *et al.*, 2003) different responses to broomrape were observed.

On the other hand, different responses of host varieties can cause changes in broomrape behavior. Teimouri *et al.* (2016) reported that some sesame varieties infected to *P. aegyptiaca* could not continue their reproductive stage. Tokasi *et al.* (2014) found that the broomrape dry weight and the number of parasite stems per plant differed depending on tomato genotypes. In our study, broomrape traits showed significant differences across different genotypes, and the effect of the host genotypes on parasite behavior was confirmed. In other studies, the mechanism of resistance was related to broomrape attachment necrosis, creation of physical barriers in the cortex, reduced stimulation of germination, and increase in phenolic compounds and peroxidase activity in the host plant (Zahhar *et al.*, 2003; Buschmann *et al.*, 2005). In addition, other factors can influence the host-parasite interaction, such as changes in agricultural practices (Grenz *et al.*, 2005; Haidar and Sidahmed, 2003, 2006; Labrousse *et al.*, 2010; Mahgoub *et al.*, 2012) or climate conditions (Teimouri *et al.*, 2016).

The importance of the underground stage of the parasite was confirmed in our results and showed the importance of the total number of attachments per plant (TAN). In contrast, the amount of emergence *P. aegyptiaca* per plant had no significant relation to root dry weight loss percentage of cucumber in our experiment. Teimouri *et al.* (2016) showed that there was a positive correlation between host roots and *P. aegyptiaca* dry weight. In contrast, Mauromicale *et al.* (2008) reported that there was no direct correlation between these two traits. Indeed, our results showed no significant correlation between shoot dry weight loss percentage and *P. aegyptiaca* traits, which indicates that the intensity of *P. aegyptiaca* effects on cucumber has no relation to its number of attachments per plants. Mauromicale (2008) believed that the cause of a decrease in shoot dry weight was damage to the photosynthetic system and the

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disconnection of shoot and root, as well as the imbalance of hormones like ABA (Taylor *et al.*, 1996; Jiang *et al.*, 2010). Damage to the photosynthetic system was confirmed in our results by the decrease in chlorophyll content, *fv/fm* and the photosynthesis rate in all cucumber genotypes. It is worth noting that a low decrease in *fv/fm* rather than in other traits can be attributed to some inhibition in the reaction center of PSII in treated plants. This case has also been reported by Stepien and Klobus (2006) in cucumbers under stress conditions.

The direct result of a reduction in photosynthesis is the decline in growth and effect on phenotypic traits, including a reduction in leaf number and leaf area. However, there is no direct relationship between the increased parasite attack and host shoot dry weight (Mauromicale *et al.*, 2008).

Based on cluster analysis, it was determined that the photosynthesis rate and maximum quantum yield of PSII chemistry (which indicates susceptibility to photo-inhibition) played an important role in the response of genotype to broomrape. With a lower decrease in dry shoot weight, genotype 22 was able to prevent damage to the photosynthesis system to some extent. In the studies by Graves *et al.* (1989) on sorghum and Mauromicale *et al.* (2008) on tomatoes, the reduction in carbon assimilation was the most important factor in the amount of parasite damage to host plant, which had been initially reduced. This can be attributed to the decrease in root volume and the relationship between root and shoot. Also, despite the high UAN, TAN and ADW, the attribute of ESN in genotype 22 was the lowest of all the other genotypes tested. The different behavior of this genotype makes it a good candidate for future research to elaborate on the sources of plant resistance.

It should be noted that a comprehensive evaluation of the damage and interaction between the host plants and parasites should be further studied. Further, the study of physiological and morphological responses and the identification of effective traits in each host would provide a better understanding of the host interactions and could be effective in finding resistant varieties or adopting effective control methods.

Conclusion

Our results showed a high sensitivity of cucumber genotypes to *P. aegyptiaca*. There was also a variation between the genotypes in their responsiveness to parasitism and their effects on the parasitic plant. Moreover, genotype 22 had different behavior compared to the other genotypes, with the lowest decrease in shoot dry weight and total broomrape attachment number per plant. The information gathered here could be used by plant breeders, though no cucumber genotype emerged sufficiently tolerant of *P. aegyptiaca* parasitism. Further selection within superior plant lines and identification of suitable traits will be necessary to provide improved planting material to farmers.

Abbreviations used:

UAN: underground attachments number plant^{-1}.
ESN: emerged spikes number plant^{-1}.
TAN: total attachment number plant^{-1}.
ADW: dry attachment weight (g) plant^{-1}.

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بررسی پاسخ‌های فیزیولوژیک و مورفولوژیک خیار *Cucumis sativus* به انگلی‌شدن گل جالیز *Phelipanche aegyptiaca*

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چکیده: به‌منظور بررسی اثر آلودگی گل جالیز مصری بر روی خصوصیات رشدی، میزان فتوسنتز، فلورسانس کلروفیل و محتوای کلروفیل برگ در خیار، آزمایش گلخانه‌ای با ۳۵ ژنوتیپ مختلف انجام شد. تقاضای بالای اسیمیلات‌ها توسط گل جالیز مصری باعث کاهش معنی‌دار وزن خشک ریشه، ساقه، ارتفاع، تعداد و سطح برگ در تمامی ژنوتیپ‌های مورد آزمایش شد. در ژنوتیپ‌های آلوده محتوای کلروفیل برگ، نرخ فتوسنتز، حداکثر کارایی کوانتومی فتوسیستم دو (f_v/f_m) به‌طور معنی‌داری کم‌تر از شاهد بدون آلودگی بود که نشان‌دهنده کاهش در اسیمیلاسیون کربن، کارایی فتوسنتز و حساسیت بیشتر ژنوتیپ‌های آلوده به بازداشت نوری می‌باشد. خصوصیات اندازه‌گیری شده در گل جالیز به‌طور معنی‌داری تحت تأثیر ژنوتیپ‌های خیار قرار گرفت. ارتباط معنی‌داری بین خصوصیات اندازه‌گیری شده گل جالیز و درصد کاهش وزن خشک اندام هوایی و ریشه نبود. بین تعداد اتصال گل جالیز در زیر خاک (UAN) و درصد کاهش وزن خشک ریشه خیار (۰/۵۸-) و هم‌چنین بین تعداد کل اتصال به‌ازای هر گیاه (TAN) و درصد کاهش وزن خشک ریشه (۰/۳۹-) ارتباط معنی‌دار دیده شد. برطبق نتایج به‌دست آمده ژنوتیپ‌های خیار به سه دسته تقسیم گردید و برمبنای این دسته‌بندی‌ها، ژنوتیپ شماره ۲۲ (خسیب) رفتار متفاوتی نسبت به دیگر ژنوتیپ‌ها داشته و از آلودگی گل جالیز آسیب کم‌تری دید.

واژگان کلیدی: سرعت فتوسنتز، فلورسانس کلروفیل، گیاه انگل، محتوای کلروفیل

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