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ORIGINAL ARTICLE

Physiological and growth responses of cucumber (*Cucumis sativus* L.) genotypes to Egyptian broomrape (*Phelipanche aegyptiaca* (Pers.) Pomel) parasitism

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Abstract
Lack of consensus on effective approaches for Egyptian broomrape (*Phelipanche aegyptiaca*) management in host plants has focused attention on the identification of resistant or tolerant genotypes by evaluating plant physiological responses to parasitism. In the current study, the response of 35 cucumber (*Cucumis sativus* L) genotypes to Egyptian broomrape parasitism was investigated in a completely randomized design with 4 replications. Parasitism by Egyptian broomrape led to a significant decrease in shoot and root dry weight of *C. sativus* compared to the control treatment which varied across different genotypes. Parasitism resulted in significant but variable increases in hydrogen peroxide, malondialdehyde, protein and phenolic compound content across all genotypes. Catalase-specific activity, ascorbate peroxidase and peroxidase in Egyptian broomrape-infected treatments also differed to that in control treatments. In addition, 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) were significantly different among various cucumber genotypes. As a result of our analysis, genotypes were classified into four clusters. The Khassib greenhouse genotype was different to other genotypes with increases in total phenolic compound content and catalase activity being higher compared to the other three clusters. At the same time, shoot dry weight losses, malondialdehyde enhancement and UAN traits in Khassib had the lowest values compared to the other genotypes when parasitized by broomrape.

Keywords Antioxidant enzymes · Host resistance · Oxidative stress · Phenolic compounds

Introduction

Some specific traits of weedy parasite Egyptian broomrape including its close association with the host and its effect on physiological processes, imposing damage on host during parasite underground developmental stage (Rubiales 2003), dependence on presence of host for germination (Boyer et al. 2014), long seed viability, high infestation rate, high seed production (Samejima and Sugimoto 2018) and potential of developing a strong seed bank in the short term (Velasco et al. 2000) have led to the failure of conventional control and management methods.

It is reported that host resistance to the parasitic weeds and adopting resistant cultivars are the most important and effective integrated weed management method (Labrousse et al. 2001; Scholes and press 2008; Das et al. 2020). Buschmann et al. (2005) found that spring and winter oilseed rapecultivars had different susceptibility to *O. ramose* and even significant yield loss was observed in cultivars with low susceptibility (58%). In maize, thicker xylem, faster cell division in the vascular region and the formation of an impenetrable layer have caused the maize genotypes to be completely resistant to Egyptian broomrape (Zahar et al. 2003). Various investigators have reported an increase in the amount of phenolic acids in resistant hosts compared to the susceptible ones.

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In vetch and parsley, an increase in the phenolic compounds content has been observed in treatments infected with Egyptian broomrape (Goldwasser et al. 1999, 2000, 2002). Pérez-de-Luque et al. (2005a, b) also reported an increase in total soluble phenolic compounds content in some chickpea genotypes. According to this, the activity of peroxidase enzymes such as guaiacol peroxidase was also higher in resistant chickpea genotypes than in susceptible ones. González-Verdejo et al. (2006) also was noted that increased peroxidase activity in the root of the parasite causes faster host infestation and overcomes reactive oxygen species (ROS) accumulation at the site of infestation. These researchers pointed out that peroxidases play an important role in the production of extracellular ROS to destroy the host cell wall, which accelerates the elongation parasite root.

Hydrogen peroxide (H_2O_2) accumulation in response to infestation by *O. crenata* has also been reported (Pérez-de-Luque et al. 2006; Mabrouk et al. 2007). All forms of reduced oxygen, including superoxide ($O2^{o-}$), hydroxyl radical ($^{\cdot}OH$) and H_2O_2 are known as ROS. These species are made under stress conditions and initiate plant defense reactions. The accumulation of reactive oxygen species leads to oxidative stress that can damage cell components such as membranes, proteins and DNA (Hsieh et al. 2002; Verma and Dubay 2003). Among the ROS, hydrogen peroxide is more dangerous because it can pass through the membrane and reach intracellular organelles. Thus, the balance between production and elimination of H_2O_2 is essential for plant survival. On the other hand, the accumulation of reactive oxygen species in the host also reflects the reaction of the plant to the presence of the parasitic plant and faster accumulation of H_2O_2 can means faster initiation of defense response and subsequence more resistance (Mor et al. 2008; Torres et al. 2010).

To counteract oxidative stress, the plant subjected to stress adopts two different defense mechanisms including enzymatic (including catalase, peroxidase, superoxide dismutase, glutathione reductase, glutathione peroxidase (Hsieh et al. 2002) and non-enzymatic mechanisms (flavonoids and anthocyanins, carotenoids, vitamin E, and alkaloids (Shahid 2014)) to neutralize free radicals and prevent them from damaging the cell.

Among the effective enzymes, catalase saves as a high efficiency catalyst with high energy use efficiency and is superior to other enzymes (Sharma 2013). The importance of these enzymes in coping with stress is reported in the literature (Bočová et al. 2012; Youssef and Azooz 2013). According to the results of González-Verdejo et al. (2006), the exogenous application of catalase due to H_2O_2 degradation inhibits the parasite weed growth. The researchers found that catalase increased activity inhibited elongation of root of the parasite.

The cucumber is one of the most important fruit vegetables in the world and Iran (FAO 2019). It was reported that melon and watermelon species as major hosts of the Egyptian broomrape in the *Cucurbitaceae* family (Joel et al. 2013). However, there is evidence of a change in parasitic plants host so that repeated cultivation and monoculture of a crop can lead to host change (Olivier et al. 1998). In greenhouses and farms in Iran, monoculture or rotation of tomato–cucumber cultivation are carried out. According to the reports and the observations of the authors, the presence of this parasite in greenhouses and farms of cucumber in Iran has incurred considerable damage to this crop. Given that Iran occupies the second place in terms of cucumbers production with an average annual production of 1.981.130 tons in Asia (FAO 2019), the study of yield-reducing factors in this crop is necessary.

In this study, we investigated the effects of physiological and growth responses of 35 conventional commercial cultivars and native Iranian cucumber genotypes to Egyptian broomrape parasitism. We studied the change in antioxidant enzymes (APX, POX, CAT) activities, lipid peroxidation and hydrogen peroxide level and some growth parameters in these genotypes. The topic is novel for cucumbers and selection of superior genotypes is useful in the development of integrated weed management in cucumber production and for guiding breeding programs.

Materials and methods

To assess physiological responses of 35 cucumber genotypes, an experimental utilizing a completely randomized design with 4 replications was conducted in the greenhouse at the Department of Agriculture, Isfahan University of Technology (51° 31′ N, 32° 43′ E) from May to July 2017. The greenhouse was constructed with a steel frame and a transparent polyvinyl chloride (PVC) coating and was maintained at 25/15 C (night/day), 65–75% relative humidity and photosynthetic active rate of 300 µmol m^{-2} s^{-1}, respectively. Genotypes included 17 native genotypes (Table 1), 8 commercial greenhouse genotypes and 10 commercial genotypes. Seeds of native cultivars were obtained from the Iran Seed and Plant Improvement Research Institute.

To evaluate the response of each genotype, eight plastic pots with 30 cm height and 25 cm diameter containing a soil-less culture of 50% fine perlite, 40% sand and 10% pumice was utilized. Four pots were sown with cucumber seeds alone, with four remaining pots sown both with cucumber and 20 mg seed of Egyptian broomrape which had been collected from infected tomato fields around Isfahan (Center of Isfahan province, Iran). After filling two-thirds of the pots in the infected treatments, 20 mg of Egyptian broomrape seed

Table 1 Cucumber genotypes considered in this study

Domestic genotypes				Greenhouse genotypes	Field genotypes
Genotype	Origin	Genotype	Origin	Genotype	Genotype
55950	Kurdistan	56013	Tehran	Storm	Baran
55952	Fars	56032	Gillan	Negin	Superdomino
55956	Yazd	56043	zanjan	Keyhan	Omid
55957	Markazi	56044	Zanjan	Alfarid	Emprator
55960	Yazd	56046	Khorassan	Khassib	Clause
55961	Azarbaijan	Kharvan	Naein	Espadana	Bingo
55963	Hamadan	Dastgerd	Isfahan	Newsun	Grifaton
55995	Mazandaran			Kaspian	Kaveh
56002	Azarbaijan				Pop
56005	Booshehr				Argeto

was mixed with culture and then were sown (El-Halmouch et al. 2006). For better germination, the seeds of Egyptian broomrape were pre-treated for one week with 30 mg/L gibberellic acid (Merck Co., Germany) at 18 °C in the dark (Song et al. 2005).

Initially, three cucumber seeds were planted in each pot and after seedling establishment, the seedlings were thinned and finally in each pot one plant of each genotype was remained. Irrigation was performed according to the crop requirement and to field capacity and pots were subjected to Hoagland and Arnon (1950) nutrient solution according to conventional nutrition schedule. At the two-leaf stage, cucumber plants were treated with Mancozeb fungicide (Dithane M45WP 80%, Gyah Co.) with irrigation. To conduct antioxidant analysis and determination of (hydrogen peroxide) H_2O_2, malondialdehyde (MDA), protein, chl *a* and *b* content and total phenolic content during the growing season and after the emergence of at least one stem of Egyptian broomrape in 90% of pots, a mature and healthy leaf from all the control and infected treatments was separated wrapped in aluminum foil, rapidly frozen in liquid nitrogen and then stored at − 80 °C until physiological measurements were undertaken.

Analysis of lipid peroxidation

As per method of Zhou and Leul (1998), lipid peroxidation was specified in terms of malondialdehyde (MDA) formation. Leaf samples were prepared by taking 0.2 g fresh leaf which were treated with liquid nitrogen, after adding 2 ml trichloroacetic acid (TCA) 0.1%. Samples were then centrifuged at 10,000*g* for 10 min at 4 °C and then extracted in 10 mL solutions of 0.25% thiobarbituric acid (TBA) and 10% trichloroacetic acid (TCA). The extract was heated in a water bath at 95 °C for 30 min and cooled down on ice immediately. After centrifugation at 5000*g* for 10 min, the absorbance was measured at 532 and 600 nm (subtracted for correction of non-specific turbidity) using a UV–Visible spectrophotometer (HITACHI, U 1800, Japan). The MDA content was expressed as μmol g^{-1} FW using an extinction coefficient of 155 $mMcm^{-1}$.

H_2O_2 analysis

To measure H_2O_2, fresh samples (0.2 g) were extracted with 5 mL of 0.1% TCA (w/v), placed in an ice bath and centrifuged at 12,000*g* for 15 min at 4 °C (Velikova et al. 2000). Then, 0.5 mL of 100 mM phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide were added to 0.5 mL of the supernatant. The absorbance was read at 390 nm and a standard curve was used to calculate the H_2O_2 content.

Protein content measurement

The method described by Bradford (1976) was used to measure total protein content of leaf samples. Bradford dye reagent contained 50 mL ethanol (95%), 100 mL orthophosphoric acid and 100 mg Coomassie Brilliant Blue diluted to make up 1000 mL. A total of 2992 μL of the Bradford reagent was added to test tubes containing 6 μL samples of the protein extract. The tubes containing the extract and Bradford reagent were subsequently incubated at room temperature for 30 min, thoroughly mixed, and the absorbencies were measured at 595 nm and compared to bovine serum albumin (BSA) as a standard.

Enzyme activity

About 0.5 g of fresh sample was homogenized in 8 mL of 50 mM potassium phosphate buffer (pH 7.8) in ice-cold mortars for enzyme analysis, and centrifuged at 14,000*g* at 4 °C for 30 min. The supernatant obtained was used for further biochemical analysis.

Peroxidase (POX-EC 1.11.1.7 extinction coefficient = 26.61 mM^{-1} cm^{-1}) activity was determined according to the method of Zhou and Leul (1998). The reaction mixture (3 mL) was composed of 50 mM potassium phosphate buffer (pH 7.0), 1% guaiacol, 0.4% H_2O_2 and 100 µL enzyme extract. Variation in absorbance due to oxidation of guaiacol was assayed spectrophotometrically (U-1800 UV/VIS, Hitachi, Japan) at 470 nm. POX activity was expressed as unit per milligram of protein (Herzog and Fahimi 1973). One unit of POX activity indicates the amount of enzyme that catalyses the oxidation of 1.0 µM of guaiacol in 1 min.

Catalase activity (CAT-EC1.11.1.6, extinction coefficient = 39.4 mM^{-1} cm^{-1}) was assayed by measuring the degradation of H_2O_2 for 1 min at 240 nm (Aebi 1984). Reaction mixture (3 mL) contained 50 mM potassium phosphate buffer (pH 7.0), 2 mM EDTA-Na2, 10 mM H_2O_2 and 100 µL enzyme extract. CAT activity was expressed as unit per milligram of protein (Chance and Maehly 1955). The amount of CAT required to decompose 1.0 µM of H_2O_2 per min was defined as one unit of CAT activity.

Determination of ascorbate peroxidase (APX, EC1.11.1.11, extinction coefficient = 2.8 mM^{-1} cm^{-1}) activity was measured in a reaction mixture (3 mL) containing 100 mM phosphate buffer (pH 7.0), 0.1mMEDTA-Na_2, 0.3 mM ascorbate, 0.06 mM H_2O_2 and 100 µL enzyme extract. The change in absorption was read at 290 nm for 1 min after addition of H_2O_2 (Nakano and Asada 1981). APX activity was expressed as unit per milligram of protein (Herzog and Fahimi 1973). One unit of APX activity represents the amount of enzyme that catalyses the oxidation of 1.0 µM of ascorbate in 1 min.

Phenolic compound determination

Total phenolic compounds were determined according to the method of Kofalvi and Nassuth (1995). For this purpose, 0.1 g fresh leaf was homogenized by 5 mL ethanol 95% after putting samples at 25° C for 24 h, 1 mL ethanol 95%, 4 mL distilled water, 1 mL $NaCO_3$ and 0.5 mL Folin's reagent was added to 1 mL of sample. Phenolic compounds concentrations were measured using p-coumaric acid as a standard at 725 nm.

Measurement of chlorophyll content (Chl *a* and *b*)

To gauge the content of Leaf Chl *a* and *b*, 0.3 gr of fully expanded healthy leaves were ground as a sample, the extract purified with 10 mL of 80% (v/v) acetone (Lichtenthaler and Wellburn 1983), and absorbance measured at 646.8 nm and 663.2 nm to quantify Chl *a* and *b* using Eqs. 1 and 2:

$$\text{Chl}a\,(\mu g/ml) = 12.25\,A_{663.2} - 2.79\,A_{646.8} \quad (1)$$

$$\text{Chl}b\,(\mu g/ml) = 21.50\,A_{646.8} - 5.10\,A_{663.2} \quad (2)$$

That Chl*a* and Chl*b* are the content of chlorophyll *a* and *b*, and A is the absorbance in mentioned wavelength, respectively.

Shoot and root dry weights of cucumber were measured at the end of the experiment and after plant ripening (90 days after planting). For this purpose, the plant was separated from the crown and placed in appropriate pocket and then transferred to the oven at a temperature of 70 °C. After 4 days, the sample was weighed with a digital balance with precision 0.01 g. Before drying the roots in treatments infected with Egyptian broomrape, necrotic nodes and attachment were isolated. The measured traits related to Egyptian broomrape in infected treatments included underground attachments number/plant (UAN), emerged spikes number/plant (ESN), total attachment number/plant (TAN), attachment dry weight (g)/plant (ADW). To determine the attachment number of Egyptian broomrape underground, root washing method in fine mesh sieve was used. The total number of Egyptian broomrape attachments was calculated from the sum of the measured values of UAN and ESN traits. Total dry weight of Egyptian broomrape attachments was also measured after drying the total parasite attachment the method used for dry weight of cucumber genotypes.

Data analysis

For data analysis according to the purpose of the experiment and based on the method of Mauromicale et al. (2008), for each trait, the change percentage of the trait in infected genotypes compare to the control was calculated. Generalized linear models employed in PROC GLIMMIX of SAS (version 9.4; SAS Institute, Gary, NC) were used to analysis the effect of treatments on response variables. The least squared means (LSMEANS) statement of the GLIMMIX procedure in SAS was used to compare treatment means at the 5% level of significance according to Fisher's least significant difference (Fisher's LSD). According to the Shapiro–Wilk test, no statistical transformation was necessary. Also, to better understand the conditions of the experiment, the measured traits of each genotype in the non-contaminated Egyptian broomrape condition are given along with the change percentage of each trait.

To classify cucumber genotypes, multivariate statistical analysis and classification methods were used by cluster analysis. For this purpose, the matrix of similarity was calculated and, using between-group linkage and squared Euclidean distance measurements, a dendrogram was drawn in to cucumber cultivars. The Pearson correlation coefficient between Egyptian broomrape traits and

the percentage of cucumber shoot and root loss were also measured.

Results

Shoot dry weight

Egyptian broomrape infection significantly reduced shoot dry weight relative to untreated controls (Table 2, $p \leq 0.01$). The shoot dry weight of all infected genotypes was significantly reduced from 55.7 to 91.7% relative to untreated controls. Shoot dry weights were reduced by between 55.7% (Khassib) and 91.7% (55952) of the control (Table 3).

Root dry weight

Root dry weights of cucumber was significantly ($p \leq 0.01$) affected by Egyptian broomrape. The lowest root damage was observed in Khavaran (− 44.5%) followed by Kaspian (59.5%), Kaveh (42.7%), Omid (78.4%), 56032 (80.7%), 55963 (82.1%) and Emperator (83.2%) genotypes (Table 3). Egyptian broomrape reduced root dry weight by greater than 95% in genotypes 55956 (95.1%), 55960 (95.2%), 55995 (95.0%), 56013 (95.5%), 55950 (96.4%), 56043 (96.4%), Pop (96.6%), Khassib (97.6%) and Alfarid (98.0%) (Table 3).

Chlorophyll *a* and *b* content

Chlorophyll *a* and *b* content in all tested cucumber genotypes was seriously affected by broomrape infection (Table 3). The percentage decrease in chlorophyll *a* and *b* content varied between 19.2 (Kaveh) and 68.6% (Clause).

Malondialdehyde (MDA) content

Malondialdehyde content was significantly increased in all genotypes as a result of Egyptian broomrape infections (Table 4). Greatest increases in malondialdehyde were in the genotypes 56002, 56032, 55995, 55952, Emperator and Storm, with an increase of 416.6–833% and the lowest percentage of changes in malondialdehyde content were observed in genotypes 56043 (61.1%), Newsun (65.8%) and 55961(76.5%) compared to control treatments (Table 4).

Hydrogen peroxide (H_2O_2) content

Egyptian broomrape infection significantly increased hydrogen peroxide content in hydrogen peroxide content in all cucumber genotypes (Table 4). Hydrogen peroxide content varied from 86.1% (55960) to 121.1% (55961). Greatest increases in H_2O_2 were associated with genotypes Baran (659.8%), 56013 (616.8%), Pop (556.7%), Bingo (542.3%), Grifaton (518.6%) and Kaspian (511.3%) genotypes (Table 4).

Protein content

Egyptian broomrape significantly increased the soluble protein content in all genotypes (Table 4). Increases in protein content was significantly increased from 271.6% in genotype 55961 followed by 559,571 (185.9%), 55960 (177.3%), 55956 (157.8%) and Superdomino (154.4%) when compared to untreated controls. Differences were not statistically significant for genotypes 55952 (18.7%), Keyhan (20.8%), Newsun (21.2%), Kaveh (24.2%), Alfarid (32.4%), 55950 (42.0%), 56032 (42.5%), POP (42.6%) and 55956 (46.0%).

Phenolic compounds content

Phenolic compound content increased in all genotypes compared to the control treatment as a result of Egyptian broomrape infection. Greatest increases were observed in Baran (63.6%) and Clause (83.6%) genotypes while lowest increase in phenolic compounds content were recorded in genotypes 55995 (3.5%), 56005 (3.5%), Kaveh (4.6%) and 55963 (5.5) (Table 4).

Activity of peroxidase

The specific activity of peroxidase in the genotypes 55961, 555957 and 55956 in the infected genotypes was lower compared to controls. The lowest increase in specific activity of the enzyme was observed in the Negin genotype due to infection with Egyptian broomrape. In this genotype, the enzyme activity was 1.2% higher than the control. The highest peroxidase activity was observed in Griffaton (497.8%), 56032 (448.9%), Keyhan (424.4%), Dastgerd (396.9%) and Alfred (393.9%) genotypes (Table 5).

Activity of catalase

Catalase activity in infected genotypes was reduced relative to controls (Table 4). Catalase activity was increased in infected genotypes 55963, Kaspian, 55956 Negin, Griffaton

Table 2 Means of square of variance analysis in different cucumber genotypes traits

Source of variation	Residual	Genotype	Total
Degree of freedom (df)	105	34	139
MDA (percentage of changes)	53829.26	111893.2**	68031.95
Change percentage of H_2O_2 (percentage of changes)	35793.31	79001.27**	46362.17
Change percentage of protein (percentage of changes)	772.2234	11729.3**	3452.372
Peroxidase activity (percentage of changes)	10954.96	84560.2**	28959.12
Catalase activity (percentage of changes)	16980.47	288908**	83495.12
Ascorbate peroxidase activity (percentage of changes)	125764.5	1221766**	393851.2
Phenol (percentage of changes)	323.3936	1138.06**	522.6645
Shoot dry weight (percentage of changes)	29.34	313.11**	75.50
Root dry weight (percentage of changes)	233.7661	727.9187**	314.1546

*, **, ns are significant different in 0.05, 0.01 levels and non-significant different, respectively

and 5604 by 30.7, 21.0, 19.0, 14.9, 7.4 and 0.5%, respectively. Considering that catalase activity in all infected genotypes was higher than the control genotypes (data not shown), it can be concluded that the final index value in these genotypes was due to protein content. Greatest increases in catalase activity relative to controls was 738.4, 755.9, 765.9 and 894.0 percent in Alfarid, 55995, 55950 and 55960 (Table 5).

Ascorbate peroxidase activity

Other than Omid genotypes (− 28.3%) and 56013 (− 20.1%), the activity of ascorbate peroxidase in genotypes infected with Egyptian broomrape was higher compared to control genotypes. The lowest change in activity of ascorbate peroxidase enzyme was found in genotypes 55960 and 56043 (Table 5). In these two genotypes, the activity of this enzyme in infected genotypes was 0.1% and 21.4% more than control, respectively. The highest increase in enzyme activity was related to Newsun genotype with 3045.9% followed by Superdomino (1228.2%) and 55952 (838. 5%). In the Newsun and Superdomino genotypes, the initial activity of the enzyme in the control treatment was very low, however, the rate of changes in the infected genotype was very high compared to the control.

Egyptian broomrape-related traits

Egyptian broomrape-related traits in infected treatment differed significantly ($p \leq 0.01$) across 35 cucumber genotypes. Results showed that the lowest mean of emerged spike number (ESN) was related to genotype 55961 (5.33 stem per plant per pot) compared to genotypes Dastgered (5.83), 56032 (6.0), Clause (6.5), 55952 (6.66), Omid (6.83), 56002 (7.83), Bingo (7.0), Superdomino (7.5), 55950 (7.66), Kharvan (7.66), 56044 (7.83), 56005 (7.83), Kaveh (7.83) and Baran (7.83 stem plants per pot). The highest number of ESN was observed in genotype 55995 (16.5) followed by Negin and 56046 genotypes with 13.66 emerged spikes per pot. The mean ESN in the other genotypes ranged from 8.16 to 10.66 and were not statistically different (Table 6).

Underground attachments number per plant (UAN) was varied significantly among cucumber genotypes (Table 6). The highest UAN was related to Kaveh and Kharvan genotypes with an average of 16.33 and 13.66 attachments per plant, respectively. The lowest UAN of Egyptian broomrape was related to Dastgerd with a mean of 0.33.

Total attachment number per plant (TAN) also varied across cucumber genotypes. The highest number of attachments was recorded for Kaveh (24.61), 56046 (23.0), Kharvan (21.3) and 55995 (20.8) genotypes. The lowest number of attachments were related to Dastgerd (6.5), 56002 (10.2) and Clause (10.3) genotypes.

Attachment dry weight (g) per plant (ADW) in the tested genotypes ranged from 0.63 to 2.18 g. The highest and the lowest total attachment dry weight (ADW) of Egyptian broomrape was recorded for Clause (2.18 g) and 55952 (0.63 g). (Table 6).

Classification of cucumber genotypes by cluster analysis

Cluster analysis (based on Euclidean Distance) was used to place genotypes into four clusters based on all measured traits (Fig. 1).

Cluster I: Kharvan, Kaveh and Kaspian genotypes.

Cluster II: Newsun, 56013, Alfarid, Dastgerd, Superdomino, Argeto, Spadana, 55952, 55950, 55956, 55995, Negin, 55963, Storm, 56044 and Griffaton genotypes.

Cluster III: Khassib genotype,

Table 3 Mean comparison of percentage of changes of phenol, Shoot and Roor dry weight and Chlorophyll *a* and *b* content

Genotype	Shoot dry weight		Root dry weight		Chl *a* + *b*	
	Noninfected plant (g)*	%Change ± SE	Noninfected plant (g)	%Change ± SE	Noninfected plant (µg/ml)	%Change ± SE
55950	16.85	− 86.88 ± 1.34	7.20	− 96.42 ± 0.22	31.11	− 27.66 ± 1.23
55952	18.57	− 91.65 ± 1.01	4.98	− 95.40 ± 0.30	22.34	− 39.18 ± 0.75
55956	18.32	− 84.14 ± 1.73	4.27	− 95.14 ± 0.70	20.07	− 34.88 ± 2.84
55957	18.05	− 88.70 ± 0.96	2.21	− 94.42 ± 0.77	18.83	− 61.07 ± 1.93
55960	17.39	− 77.57 ± 2.20	3.45	− 95.15 ± 1.49	17.51	− 60.73 ± 2.22
55961	18.30	− 81.32 ± 5.89	5.53	− 89.32 ± 3.67	37.94	− 46.62 ± 1.96
55963	15.16	− 85.39 ± 1.99	5.01	− 82.05 ± 10.51	21.65	− 37.26 ± 1.20
55995	17.95	− 87.47 ± 0.54	4.92	− 95.03 ± 1.49	17.03	− 38.32 ± 1.70
56002	18.22	− 87.72 ± 0.69	3.92	− 94.31 ± 2.01	26.28	− 62.33 ± 0.59
56005	16.74	− 82.87 ± 1.26	4.81	− 92.86 ± 1.42	32.96	− 68.64 ± 1.48
56013	16.80	− 87.15 ± 0.69	5.98	− 95.50 ± 0.48	21.68	− 22.00 ± 0.08
56032	17.18	− 89.10 ± 0.44	1.86	− 80.66 ± 2.63	21.72	− 58.28 ± 0.30
56043	13.00	− 83.31 ± 1.48	5.66	− 96.37 ± 0.58	28.75	− 48.94 ± 0.63
56044	12.12	− 85.41 ± 0.99	5.01	− 91.11 ± 1.96	22.07	− 42.14 ± 1.60
56046	14.12	− 87.72 ± 1.10	6.51	− 92.77 ± 1.23	22.90	− 60.93 ± 1.46
Dastgerd	14.47	− 73.45 ± 1.29	4.15	− 92.82 ± 0.74	21.82	− 42.05 ± 3.82
Kharvan	14.23	− 86.61 ± 0.72	4.07	− 44.51 ± 8.21	17.86	− 34.84 ± 1.82
Omid	16.40	− 77.58 ± 3.56	5.74	− 78.40 ± 1.71	25.35	− 62.28 ± 2.00
Baran	13.91	− 82.86 ± 1.33	9.48	− 93.45 ± 0.60	23.06	− 54.43 ± 1.60
Emperator	10.85	− 90.53 ± 0.75	5.84	− 83.20 ± 5.63	15.64	− 50.24 ± 2.40
Clause	11.50	− 80.99 ± 1.28	1.60	− 87.55 ± 1.80	22.01	− 66.53 ± 1.34
Bingo	12.70	− 82.82 ± 1.23	3.88	− 90.60 ± 0.79	32.26	− 65.46 ± 1.93
Grifaton	12.30	− 87.72 ± 1.61	3.40	− 90.22 ± 3.22	28.93	− 42.80 ± 4.52
Kaveh	13.00	− 73.02 ± 2.58	2.46	− 66.42 ± 6.32	23.29	− 19.22 ± 1.15
Superdomino	12.10	− 77.16 ± 1.44	6.45	− 86.80 ± 1.91	18.93	− 40.34 ± 2.48
Pop	15.02	− 87.40 ± 1.30	6.18	− 96.63 ± 0.55	23.42	− 49.46 ± 1.01
Argeto	14.67	− 70.93 ± 2.21	3.41	− 85.16 ± 1.82	19.76	− 41.02 ± 0.86
Storm	14.50	− 80.79 ± 2.67	4.90	− 90.03 ± 1.16	17.37	− 41.23 ± 2.79
Negin	13.30	− 90.15 ± 1.30	4.91	− 90.66 ± 1.90	27.05	− 42.21 ± 2.10
Keyhan	14.00	− 82.08 ± 0.65	4.90	− 91.86 ± 2.24	33.09	− 48.91 ± 1.68
Alfarid	16.40	− 75.33 ± 1.72	5.66	− 98.01 ± 0.22	27.61	− 25.55 ± 2.46
Khassib	17.88	− 55.67 ± 2.00	6.81	− 97.63 ± 0.48	28.71	− 47.86 ± 1.32
Spadana	13.02	− 74.57 ± 3.21	4.77	− 93.95 ± 1.24	31.06	− 44.14 ± 0.99
Newsun	16.84	− 86.57 ± 3.08	3.14	− 93.29 ± 1.57	25.41	− 35.39 ± 2.11
Kaspian	15.13	− 76.23 ± 3.29	3.48	− 59.53 ± 7.00	25.69	− 27.97 ± 0.20
NIR-Fisher test		1.07×10^{-27}**		6×10^{-07}**		2.1×10^{-43}**
LSD(%5)		6.17		17.17		6.20

*In each trait, first colum is the mean value of noninfected genotype and second column is the mean comparison of percentage of changes ± standard error

Cluster IV: Omid, Clause, 56046, 56005, Bingo, 55960, 55957, 56002, 56032, Emperator, Baran, 56043, Pop, 55961 and Keyhan genotypes.

Genotypes in Cluster I had the lowest percentage of changes in catalase activity, ascorbate peroxidase and phenolic compounds and highest value of UAN and TAN compared to other clusters showing the inefficiency of the antioxidant system in this group. The small changes in root dry weight and chlorophyll *a* and *b* content were probably related to later infection. In addition, the competition

Table 4 Mean comparison of percentage of changes of MDA, H_2O_2, Protein and Phenolic compounds content of cucumber genotypes

Genotype	MDA		H_2O_2		Pr		Phenolic compounds	
	Noninfected* Plant (µmol. gr^{-1})	%Change ± SE	Noninfected Plant (µmol. gr^{-1})	%Change ± SE	Noninfected Plant (µmol. gr^{-1})	%Change ± SE	Noninfected Plant (mg. gr^{-1})	%Change ± SE
55950	2.14	+239.90 ± 34.25	1.09	+234.43 ± 66.53	0.48	+42.00 ± 2.36	1.760	+18.87 ± 4.36
55952	1.77	+453.97 ± 27.06	1.46	+226.05 ± 66.15	0.86	+18.66 ± 1.49	0.769	+36.65 ± 11.76
55956	2.26	+333.82 ± 31.67	1.319	+217.44 ± 54.56	0.40	+157.80 ± 10.14	0.947	+14.15 ± 3.00
55957	1.92	+320.48 ± 67.63	1.54	+192.08 ± 26.66	0.42	+185.91 ± 9.49	0.921	+8.50 ± 2.31
55960	1.94	+351.06 ± 87.99	2.69	+86.13 ± 21.34	0.39	+177.27 ± 23.89	0.794	+20.32 ± 5.73
55961	3.04	+76.51 ± 24.58	2.37	+126.09 ± 8.98	0.37	+270.61 ± 19.35	1.032	+20.24 ± 2.56
55963	2.75	+156.92 ± 30.92	1.02	+462.74 ± 81.11	0.79	+61.77 ± 1.15	1.074	+5.46 ± 1.13
55995	3.01	+508.06 ± 45.12	1.59	+462.99 ± 62.04	0.74	+45.98 ± 4.94	0.837	+3.54 ± 0.50
56002	1.19	+833.36 ± 40.39	1.42	+333.38 ± 58.72	0.34	+93.54 ± 3.39	0.814	+10.78 ± 2.55
56005	3.18	+274.78 ± 67.84	2.40	+315.82 ± 79.90	0.58	+122.34 ± 11.15	1.406	+3.54 ± 1.07
56013	2.47	+252.21 ± 76.57	1.30	+616.77 ± 71.11	0.43	+126.53 ± 1.68	0.924	+19.56 ± 7.94
56032	1.54	+615.59 ± 46.04	1.93	+188.04 ± 46.17	0.59	+42.45 ± 1.57	0.981	+6.22 ± 1.87
56043	5.00	+61.13 ± 4.64	1.90	+283.01 ± 60.14	0.59	+74.90 ± 3.89	0.779	+8.53 ± 2.35
56044	2.15	+345.64 ± 33.90	1.59	+340.25 ± 61.00	0.60	+93.92 ± 3.63	0.879	+10.36 ± 2.11
56046	2.70	+180.34 ± 36.58	1.89	+262.19 ± 79.25	0.63	+84.56 ± 4.03	0.971	+15.29 ± 3.51
Dastgerd	3.24	+205.21 ± 63.51	1.67	+342.47 ± 75.87	0.52	+80.36 ± 8.19	0.925	+35.98 ± 11.37
Kharvan	1.51	+343.16 ± 69.02	1.58	+220.73 ± 42.19	0.52	+107.88 ± 19.57	0.770	+32.23 ± 8.92
Omid	3.02	+114.75 ± 15.04	1.67	+328.14 ± 86.47	0.60	+63.25 ± 12.98	0.926	+30.89 ± 8.19
Baran	2.48	+311.79 ± 63.39	0.44	+659.75 ± 81.70	0.47	+88.02 ± 4.42	0.704	+63.57 ± 15.93
Emperator	1.75	+416.56 ± 89.62	1.21	+376.21 ± 53.53	0.66	+73.89 ± 4.33	0.859	+32.81 ± 5.42
Clause	3.81	+104.96 ± 43.81	1.20	+335.98 ± 71.11	0.50	+128.99 ± 30.19	0.881	+83.56 ± 15.47
Bingo	2.64	+164.36 ± 32.77	0.94	+542.31 ± 25.71	0.60	+84.44 ± 26.83	1.063	+23.16 ± 3.39
Grifaton	1.73	+293.77 ± 58.54	1.19	+518.55 ± 58.77	0.76	+59.83 ± 8.66	0.991	+17.60 ± 6.21
Kaveh	3.14	+126.02 ± 34.48	1.56	+308.92 ± 21.42	0.64	+24.21 ± 1.40	0.874	+4.64 ± 1.97
Superdomino	2.61	+214.90 ± 27.88	1.41	+455.92 ± 36.29	0.51	+154.37 ± 15.08	0.773	+44.89 ± 9.81
Pop	2.83	+113.41 ± 14.76	1.08	+556.65 ± 20.15	0.64	+42.61 ± 5.99	0.864	+23.13 ± 3.37
Argeto	3.51	+120.67 ± 15.33	1.54	+360.26 ± 64.69	0.43	+80.79 ± 5.72	0.923	+20.20 ± 2.70
Storm	1.94	+436.33 ± 49.30	1.32	+391.83 ± 19.23	0.79	+76.28 ± 11.39	1.136	+15.61 ± 5.02
Negin	2.45	+223.31 ± 45.25	1.05	+410.51 ± 98.51	0.60	+108.81 ± 25.77	0.830	+36.58 ± 12.66
Keyhan	2.99	+123.49 ± 27.04	1.48	+242.54 ± 44.39	0.89	+20.80 ± 0.78	0.908	+8.49 ± 2.94
Alfarid	3.49	+278.88 ± 26.06	2.74	+151.24 ± 34.97	0.93	+32.42 ± 7.56	1.133	+15.76 ± 5.29
Khassib	2.87	+126.75 ± 17.96	1.48	+340.13 ± 31.76	0.71	+67.32 ± 6.23	0.908	+26.79 ± 9.11
Spadana	3.48	+117.00 ± 14.98	1.90	+422.43 ± 29.76	0.66	+107.45 ± 11.75	0.929	+21.58 ± 6.01
Newsun	3.02	+65.75 ± 14.43	1.25	+495.08 ± 21.38	0.73	+21.20 ± 2.11	1.007	+21.48 ± 7.28
Kaspian	2.22	+239.75 ± 16.32	1.20	+511.25 ± 22.72	0.43	+131.91 ± 4.05	1.024	+10.19 ± 2.80
NIR-Fisher test		25.13×10^{-4}**		11.73×10^{-4}**		2.66×10^{-27}**		4.3×10^{-07}**
LSD(%5)		325.30		265.25		38.96		25.21

*In each trait, first colum is the mean value of noninfected genotype and second column is the mean comparison of percentage of changes ± standard error

between individual broomrape on host-limited resources caused the lowest ADW in this cluster (Table 7).

The genotypes in second cluster had the highest increase in H_2O_2 was associated with the highest values of broomrape traits (excepting UAN). The increase in activity of ascorbate peroxidase in this cluster was greater compared to the other three clusters. However, the activity of this enzyme could not prevent the damage of Egyptian broomrape so

that genotypes in this cluster had the high damage at all measured traits.

Cluster III comprised a single genotype (Khassib) and was characterized by the lowest values for malondialdehyde as an index of lipid peroxidation. However, the response to oxidative stress was better than the other clusters due to increase of catalase activity and total phenolic compounds content.

In cluster IV, the highest percentage of losses in MDA content was recorded. Despite the highest increase percentage of protein and peroxidase content, the percentage of shoot dry weight and chlorophyll *a* and *b* content were higher compared to other clusters. In addition, reductions in shoot dry weight and UAN were lower than the other two groups. It can be concluded that increasing in catalase activity and phenol compounds content in cucumber genotypes caused less damage from Egyptian broomrape. The amount and severity of contamination will also be reduced by increasing phenolic compounds content and catalase activity.

Discussion

Many researchers have investigated the parasitic effect of Egyptian broomrape on different hosts. The different response to parasite plants in these studies helps to classify hosts based on tolerance, susceptibility and resistance and to find sources of resistance. However, the results of different experiments on the same host are not always replicated. For example, Labrousse et al. (2001) and Ruso et al. (1996) have obtained different results from *H. argophyllus* species, which is attributed to Egyptian broomrape race or crop accession. In this experiment, cucumber genotypes responded differently to Egyptian broomrape parasitism. The effect of Egyptian broomrape could be clearly observed in all studied traits, however, the highest damage was observed in root dry weight, with a reduction between 46.5 and 98%.

In various experiments, root dry weight loss was recorded in susceptible hosts. In a study by Labrousse et al. (2001), which investigated the resistance of sunflower to Egyptian broomrape, severe root dry weight loss was observed in the susceptible cultivar, which was attributed to the intense competition between Egyptian broomrape and sunflower. The shoot dry weight losses in different genotypes of cucumber ranged from 55.7 to 91.6%. This significant reduction in shoot dry weight in the cucumber genotypes caused the yield of all infected genotypes to be zero. As a result, the fruits became yellowish in the early stages of production and fall.

Damage to the plant production systems has also been observed in other studies. A 50% reduction in sunflower biomass was reported by Labrousse et al. (2001) as a result of infestation with Egyptian broomrape. This decrease in plant biomass led to an increase in the parasite plant biomass. Barker et al. (1996) reported a fourfold reduction in shoot biomass ratio (stem, leaf and fruit pedicle) relative to root weight in infected tomatoes which indicates more severe root damage than the shoot. However, in the work of Hibberd et al. (1998), it was reported that Egyptian broomrape stimulated root growth in resistant tobacco genotypes. Furthermore, the results of Trabelsi et al. (2016) showed in *O. foetida*-infected fields, grain yield losses were between 55.7 and 83% for the breeding line compared to 97% for the susceptible cultivar.

According to our results, the decrease in chlorophyll content could be caused by broomrape infection. This has been previously confirmed by Demirbaş and Acar (2017) and Mauromicale et al. (2008). Demirbaş and Acar (2017) showed decreased in total chlorophyll and carotenoind amount via parasite infection.

In our study, cucumber greenhouse genotypes had less effect on shoot dry weight. Although the percentage of root dry weight reduction was very high in some genotypes such as Khassib, plants were able to reduce the effects of this reduction to some extent. The reason for this may is not clear exactly how much of the root organism is the root of the activity and during which part of the growing season. Since root measurement was done at the end of the growing season and there is likely to be a drying up of the active parts of the root as a result of infestation with Egyptian broomrape during the growing season (Losner-Goshen et al. 1998; Zhou et al. 2004). Thus, despite the association between shoot and root dry weight, it is possible to have the same reduction trend in the genotypes.

In other patho-systems, such as faba bean- *O. crenata*, the highest cost of growth of the Egyptian broomrape biomass was related to the reproductive and root system (Manschadi et al. 1996). The researchers noted that despite the significant reduction in biomass accumulation in the infected compared to the noninfected host and also, no difference in photosynthetic activity, the allocation of dry matter to the patho-system depends on the developmental stage of the host and the parasite. They also stated that when the germination of *O. crenata* coincided with faba bean flowering, the host was unable to produce any pods with seeds. It is assumed that Egyptian broomrape serves as an additional sink for host-derived assimilates, causing damage to its photosynthetic capacity, which in turn reduces shoot and root biomass. However, because the parasite plant is not a large sink for carbon assimilates, in most cases, the total biomass of the parasite and the infected host is significantly lower than that of the noninfected host biomass (Barker et al. 1996).

Increase in hydrogen peroxide and malondialdehyde in all cucumber genotypes due to infestation with Egyptian broomrape indicates severe oxidative stress. Activity

Table 5 Mean comparison of percentage of changes of POX, CAT and APX activity

Genotype	POX activity		CAT activity		APX activity	
	Noninfected Plant (Unit mg^{-1}protein)	%Change ± SE	Noninfected Plant (Unit mg^{-1}protein)	%Change ± SE	Noninfected Plant (Unit mg^{-1}protein)	%Change ± SE
55950	0.187	+ 97.28 ± 3.17	0.012	+ 755.91 ± 52.30	0.172	+ 117.91 ± 5.33
55952	0.214	+ 27.16 ± 1.59	0.002	+ 765.94 ± 49.24	0.037	+ 835.47 ± 42.33
55956	0.382	− 31.89 ± 4.46	0.025	− 19.03 ± 4.06	0.088	+ 45.91 ± 9.59
55957	0.290	− 54.26 ± 1.34	0.034	+ 74.79 ± 8.11	0.067	+ 87.96 ± 13.24
55960	0.051	+ 232.16 ± 29.06	0.010	+ 738.39 ± 67.97	0.200	+ 0.09 ± 0.03
55961	0.210	− 57.13 ± 2.18	0.006	+ 105.20 ± 13.56	0.042	+ 77.33 ± 14.74
55963	0.041	+ 142.53 ± 6.90	0.012	− 30.66 ± 0.41	0.046	+ 128.57 ± 34.02
55995	0.056	+ 70.77 ± 16.27	0.006	+ 218.30 ± 21.13	0.025	+ 522.90 ± 76.41
56002	0.106	+ 300.91 ± 31.15	0.032	+ 297.07 ± 3.86	0.123	+ 36.71 ± 1.83
56005	0.103	+ 109.27 ± 13.20	0.017	+ 90.37 ± 12.04	0.084	+ 301.78 ± 100.31
56013	0.124	+ 145.91 ± 17.03	0.018	+ 149.52 ± 5.31	0.154	− 20.10 ± 3.59
56032	0.067	+ 448.90 ± 12.99	0.022	+ 35.69 ± 2.35	0.127	+ 43.65 ± 6.90
56043	0.080	+ 236.62 ± 16.33	0.012	+ 90.19 ± 26.31	0.146	+ 21.44 ± 3.63
56044	0.080	+ 51.51 ± 12.79	0.016	− 0.45 ± 0.18	0.061	+ 79.50 ± 19.22
56046	0.061	+ 161.74 ± 10.49	0.012	+ 36.74 ± 11.88	0.024	+ 708.80 ± 43.45
Dastgerd	0.049	+ 396.86 ± 76.91	0.006	+ 597.06 ± 145.20	0.096	+ 78.29 ± 32.45
Kharvan	0.083	+ 159.07 ± 24.42	0.031	+ 23.68 ± 3.49	0.115	+ 30.07 ± 18.10
Omid	0.075	+ 280.95 ± 35.57	0.011	+ 399.52 ± 88.98	0.153	− 28.34 ± 5.57
Baran	0.114	+ 126.05 ± 22.99	0.016	+ 360.18 ± 19.40	0.095	+ 24.94 ± 3.37
Emperator	0.069	+ 33.47 ± 7.25	0.010	+ 141.50 ± 8.72	0.024	+ 387.69 ± 46.56
Clause	0.019	+ 239.11 ± 43.96	0.008	+ 16.56 ± 5.05	0.016	+ 79.64 ± 25.83
Bingo	0.086	+ 197.06 ± 33.51	0.014	+ 3.50 ± 0.64	0.028	+ 217.94 ± 27.94
Grifaton	0.049	+ 497.75 ± 123.15	0.018	− 7.43 ± 1.32	0.024	+ 109.52 ± 32.86
Kaveh	0.084	+ 243.84 ± 28.42	0.012	+ 44.31 ± 4.09	0.027	+ 100.11 ± 6.94
Superdomino	0.070	+ 190.05 ± 80.73	0.012	+ 5.87 ± 1.04	0.013	+ 1228.19 ± 178.31
Pop	0.079	+ 193.22 ± 49.54	0.003	+ 602.18 ± 89.18	0.037	+ 321.17 ± 31.16
Argeto	0.132	+ 124.87 ± 44.58	0.011	+ 167.72 ± 60.19	0.029	+ 176.17 ± 53.14
Storm	0.092	+ 75.69 ± 15.43	0.005	+ 104.04 ± 35.55	0.031	+ 419.62 ± 60.56
Negin	0.150	+ 14.24 ± 4.22	0.023	− 14.95 ± 2.24	0.062	+ 430.25 ± 135.25
Keyhan	0.058	+ 424.44 ± 61.00	0.005	+ 146.82 ± 54.23	0.024	+ 731.85 ± 181.44
Alfarid	0.053	+ 393.95 ± 93.92	0.002	+ 894.01 ± 117.10	0.055	+ 88.21 ± 6.28
Khassib	0.123	+ 33.71 ± 3.74	0.003	+ 479.37 ± 47.08	0.031	+ 226.22 ± 46.49
Spadana	0.044	+ 341.24 ± 123.77	0.009	+ 141.08 ± 41.00	0.042	+ 30.96 ± 10.70
Newsun	0.123	+ 138.58 ± 32.12	0.009	+ 370.89 ± 28.58	0.003	+ 3045.93 ± 766.49
Kaspian	0.174	+ 44.89 ± 14.50	0.023	− 21.01 ± 4.12	0.040	+ 210.25 ± 32.57
NIR− Fisher test		2.4×10^{-16}**		2.3×10^{-29}**		8.4×10^{-20}**
LSD(%5)		146.75		182.70		497.21

*In each trait, first colum is the mean value of noninfected genotype and second column is the mean comparison of percentage of changes ± standard error

of the plant antioxidant system increasing the activity of catalase, peroxidase, phenol compounds and protein content can lead to some degree of resistance (Demirbaş and Acar 2017). Investigation of these traits in other studies has also been found to uncover sources of resistance. Some reports also suggest that the plant's usual defensive responses to the disease are also involved in response to the parasitic plants (Demirbaş and Acar 2008, 2017). Responses such as induction of phytoalexins (Serghini et al. 2001), increased peroxidase activity, lignification, and locating phenolic compounds in the cell wall (Goldwasser et al. 1999), pathogen-related proteins (Joel and Portnoy 1998) have been reported.

Table 6 Mean comparison ± standard error of Egyptian broomrape traits

Genotype	Total attachment number plant $^{-1}$ (TAN) ± SE	Emerged spikes number plant $^{-1}$ (ESN) ± SE	Underground attachments number plant $^{-1}$ (UAN) ± SE	Attachment dry weight (g) plant $^{-1}$ (ADW) ± SE
55950	11.67 ± 0.69	7.67 ± 0.65	4.00 ± 0.47	1.03 ± 0.05
55952	11.33 ± 0.38	6.67 ± 0.30	4.67 ± 0.30	0.63 ± 0.05
55956	14.00 ± 0.67	8.50 ± 0.39	5.50 ± 0.57	0.93 ± 0.07
55957	11.33 ± 0.45	8.33 ± 0.38	3.00 ± 0.53	0.93 ± 0.05
55960	14.17 ± 1.34	10.50 ± 1.17	3.67 ± 0.30	0.96 ± 0.10
55961	11.33 ± 0.90	5.33 ± 0.19	6.00 ± 0.88	0.91 ± 0.12
55963	16.67 ± 0.81	10.67 ± 0.45	6.00 ± 0.62	1.32 ± 0.16
55995	20.83 ± 1.89	16.50 ± 1.90	4.33 ± 0.56	1.76 ± 0.20
56002	10.17 ± 0.93	6.83 ± 0.64	3.33 ± 0.51	0.72 ± 0.07
56005	16.00 ± 1.05	7.83 ± 0.55	8.17 ± 1.07	1.40 ± 0.23
56013	19.00 ± 1.68	8.50 ± 0.77	10.50 ± 1.12	1.43 ± 0.17
56032	13.00 ± 1.27	6.00 ± 0.58	7.00 ± 1.03	0.81 ± 0.13
56043	14.50 ± 0.91	8.17 ± 0.80	6.33 ± 0.45	1.33 ± 0.11
56044	15.67 ± 0.38	7.83 ± 1.01	7.83 ± 0.98	1.08 ± 0.06
56046	23.00 ± 0.88	13.67 ± 0.69	9.33 ± 0.65	1.11 ± 0.07
Dastgerd	6.50 ± 1.39	5.83 ± 1.14	0.67 ± 0.30	0.92 ± 0.11
Kharvan	21.33 ± 0.81	7.67 ± 0.99	13.67 ± 1.05	1.47 ± 0.14
Omid	12.33 ± 0.69	6.83 ± 0.80	5.50 ± 0.57	1.09 ± 0.12
Baran	12.67 ± 1.31	7.83 ± 1.09	4.83 ± 0.60	1.63 ± 0.17
Emperator	19.17 ± 1.21	9.83 ± 0.96	9.33 ± 0.77	1.11 ± 0.07
Clause	10.33 ± 1.07	6.50 ± 0.81	3.83 ± 0.37	2.18 ± 0.20
Bingo	19.33 ± 1.61	7.00 ± 0.78	12.33 ± 1.43	1.52 ± 0.19
Grifaton	16.83 ± 2.20	8.67 ± 1.74	8.17 ± 0.83	1.53 ± 0.19
Kaveh	24.17 ± 2.31	7.83 ± 0.55	16.33 ± 2.12	1.05 ± 0.18
Superdomino	16.00 ± 1.08	7.50 ± 0.70	8.50 ± 1.41	1.46 ± 0.14
Pop	16.00 ± 0.91	8.17 ± 0.98	7.83 ± 0.28	1.58 ± 0.15
Argeto	17.67 ± 1.17	9.17 ± 0.64	8.50 ± 0.84	1.51 ± 0.21
Storm	15.83 ± 0.50	10.33 ± 0.73	5.50 ± 0.66	1.19 ± 0.23
Negin	19.33 ± 1.15	13.67 ± 1.52	5.67 ± 0.69	1.03 ± 0.16
Keyhan	14.33 ± 0.90	8.17 ± 0.96	6.17 ± 0.86	0.81 ± 0.05
Alfarid	12.50 ± 1.56	8.83 ± 1.12	3.67 ± 0.77	1.12 ± 0.11
Khassib	15.17 ± 1.83	9.00 ± 1.20	6.17 ± 0.80	1.17 ± 0.18
Spadana	17.00 ± 1.78	8.50 ± 0.51	8.50 ± 1.43	1.10 ± 0.12
Newsun	15.83 ± 2.07	8.67 ± 0.61	7.17 ± 1.55	1.28 ± 0.10
Kaspian	17.67 ± 1.31	8.83 ± 1.07	8.83 ± 1.01	0.90 ± 0.11
NIR-Fisher test	2.24×10^{-20} **	3.37×10^{-13} **	2.3×10^{-26} **	2.81×10^{-11} **
LSD (%5)	3.90	2.80	2.76	0.43

Westwood et al. (1998) demonstrated that parasitization of tobacco by Orabanche induces expression of a defence-related isogene of 3-hydroxy-3-methylglutaryl coenzyme A reductase which demonstrates the host resistance mechanisms to prevent parasitic contamination can occur at any stage of the host life cycle (Jorrin et al. 1999; Labrousse et al. 2001).

Angeles Castillejo et al. (2004) found that resistance in chickpea was related to the early stages of infection that accompanies with darkening of host and parasite tissues at the site of penetration and consequently the inhibition of contact with the host vascular system and the parasite development. Other studies on different hosts have shown that parasite invasion can be inhibited in the cortex (Pérez-de-Luque et al. 2006), the endoderm (Pérez-de-Luque et al. 2005a) or after reaching the pericycle. (Pérez-de-Luque et al. 2005b). In relation to Sunflower-*P. aegyptiaca*, thickening of the cell wall by suberin prevents parasite penetration

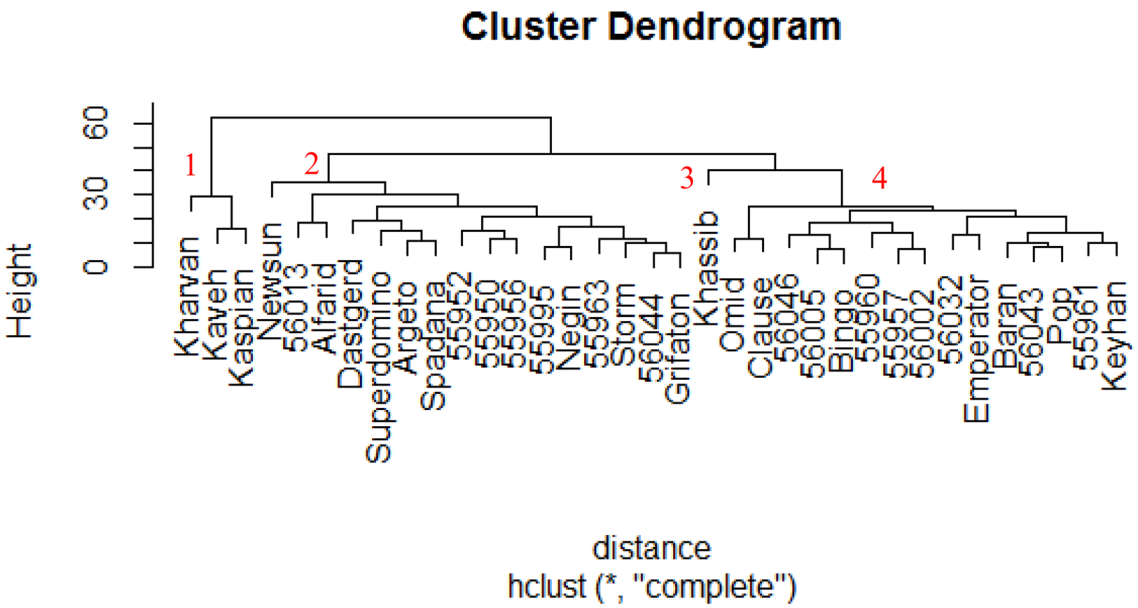

Fig. 1 Dendrogram of cluster analysis based on studied traits in cucumber using between-groups linkage. right to left: cluster I: included Kharvan, Kaveh and kaspian genotypes. Cluster II: included Newsun, 56013, Alfarid, Dastgerd, Superdomino, Argeto, Spadana, 55952, 55950, 55956, 55995, Negin, 55963, Storm, 56044 and Grifaton genotypes. Cluster III: included just Khassib genotypes and Cluster IV: included Omid, Clause, 56046, 56005, Bingo, 55960, 55957, 56002, 56032, Emperator, Baran, 56043, Pop, 55961 and Keyhan genotypes, respectively

Table 7 Mean of effective traits in determination of 35 cucumber genotypes to Egyptian broomrape infection

Traits	Cluster I	Cluster II	Cluster III	Cluster IV
Shoot dry weight	78.62	82.79	55.67	84.17
Root dry weight	57.49	91.75	97.63	90.50
Chl $a+b$	27.34	37.26	47.86	57.66
MDA	236	265	126	270
H_2O_2	346	381	340	321
Phenolic compounds	15	21	26	23
Protein	88	79	67	103
POX activity	149	167	33	191
CAT activity	15	256	479	209
APX activity	113	457	226	200
UAN	12.94	6.19	6.17	6.44
ESN	8.11	9.21	9.00	8.06
TAN	21.05	15.41	15.17	14.51
ADW	1.14	1.20	1.17	1.20

(Pérez-Vich et al. 2004). At the same time, the resistance of adjacent cells is enhanced by the production of cross-linking proteins, preventing further penetration, development and establishment of the parasite. It has also been suggested that in chickpea, resistance to *O. cerenata* caused by cross-linkage proteins (Pérez-de-Luque et al. 2006). In an experiment by Dorr et al. (1994), an isolated layer was formed around the host tissue against the penetration of *O. cumana*.

The role of peroxidases in this debate is also noteworthy (Mor et al. 2008). These enzymes are involved in the lignification and placement of phenols in the cell walls, developmental processes, defense mechanisms against pathogens and other biotic and abiotic stresses. Cross-linkage proteins and papill formation as the methods of cell wall reinforcement initiated by peroxidases in the presence of H_2O_2 soon after the pathogen attack (Bradley et al. 1992; Hammond-Kosack and Jones, 1996; Brown et al. 1998). The role of peroxidases in wall reinforcement and thus resistance to the parasitic plant has been confirmed in several patho-system (Bradley et al. 1992; Hammond-Kosack and Jones 1996; Brown et al. 1998; Caverzan et al. 2012). Increased lignification and peroxidase activity in vetch infected with Egyptian broomrape (Goldwasser 1999). In addition, increased expression of peroxidase-related genes in the process of resistance to Egyptian broomrape has been demonstrated (Vieira Dos Santos et al. 2003; Angeles Castillejo et al. 2004). Despite the role of peroxidase in resistance to Egyptian broomrape and then induction of catalase activity in host tissues another experiment has shown that peroxidase activity in both susceptible and resistant sunflower genotypes has been severely increased (Antonova and Ter Borg 1996). In addition to the cell wall subrification in sunflower, production and secretion of phyto-toxins were also observed. Phytoaloxins are phenolic compounds and considered as a protective response against *O. cumuna*.

Other studies have also shown the induction and coumarin secretion in responses to parasitism (Serghini et al. 2001). The accumulation of phenolic compounds was observed in *Vicia athropurpurea* and chickpea against *O. aegyptiaca and O. crenata* respectively (Pérez-de-Luque et al. 2005a). The secretion of these substances inhibits penetration to host tissue at the connection stage. This secretion continues until the parasite is completely stopped and the seedling dies. In addition, in resistant hosts, during the penetration phase in the cells adjacent to the parasite attack site, phenolic compounds are secreted into the apoplast and at the same time a toxic environment is created around the infestation site.

Analysis of measured traits in Egyptian broomrape showed significant differences between cucumber genotypes. It should be noted that the host has the potential to affect the traits of the parasite. In the study of Teimouri Jervekani et al. (2016), it was also shown that the traits of Egyptian broomrape are influenced by different sesame genotypes. For example, dry weight of the parasitic plant in different sesame genotypes ranged from 1.6 to 35.4% of total weight. Further, in Trablesi et al. (2016), the amount of TAN, ESN, UAN and ADW in different faba bean breeding lines was recorded. Additionally, differences in response to *O.foetida* and *O.crenata* was observed across breeding lines. In the experiment reported by Musselman (1980), there was no correlation between the degree of damage, number and biomass of Egyptian broomrape in each pot. The proposed explanation, was that the parasitic plant acts by affecting the balance of host growth related hormones through the release of toxins, and the effect of toxins is independent of the number of host-attached parasite. However, infected genotypes with higher photosynthetic rates and higher chlorophyll content are likely to be less susceptible to photo-inhibition and will be less affected. Hibberd et al. (1998) have suggested that the number of *O. cernua* attached to the tobacco root had little effect on the total dry weight of Egyptian broomrape supported by each host plant. They deduced that as the number of attachment decreases, the weight of each Egyptian broomrape spike increases as a result of the host limited assimilate production that with increase in the number of attachments and competition increases the Egyptian broomrape will be smaller.

It appears that the time of infection with Egyptian broomrape and the phenology of the host have a major influence on the type of host response. In the present experiment, simultaneous infestation of Egyptian broomrape and cucumber seed cultivation and early infection in cucumber seedling caused severe damage. Egyptian broomrape with maximum dry weight of 2.18 g severely reduced shoot and root dry weight in infected treatments. According to Mauromicale et al. (2008), the dry weight of Egyptian broomrape did not necessarily indicate the extent of damage, and the reduction in host plant biomass did not necessarily equate to increased parasite biomass. In the study of Hibberd et al. (1998) which examined the relationship between tobacco and Egyptian broomrape it had been shown that the weight of the infected plant and parasite plant was only a small proportion of the control plant without infestation. In another experiment using a 5-week tomato transplant, the shoot biomass reduction was only 39% (Mauromicale et al. 2008).

Conclusion

Genotypes of cucumber differ in response to Egyptian broomrape infestation. Egyptian broomrape infestation caused varying levels of oxidative stress and lipid peroxidation in all genotypes which triggered the enzymatic and non-enzymatic oxidative system. Although not all of the components involved in the antioxidant response were evaluated in this experiment, it appears that the role of catalase and phenolic compounds in inducing more effective responses to the parasitic plant is greater. However, it seems that while there are differences in genotype responses, cucumber is so highly sensitive to Egyptian broomrape infection, that management decisions should be focused on seed bank reduction, rotation with catch and stimulation crops, and use of agronomic methods.

Author contribution statement NHF carried out the experiment, collected samples and data, performed laboratory and chemical analyses of the samples, and prepared the first draft of the manuscript. EID, HK and AN helped in designing the experiment and reviewing the manuscript.

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