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EVALUATION OF SOME GARLIC CULTIVARS TO INFECT WITH PINK ROOT DISEASE CAUSED BY *PYRENOCHAETA TERRESTRIS* AND ITS RELATION TO SOME BIOCHEMICAL CHANGES.

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ABSTRACT: Garlic cultivars, Baledy, Seds-40, Chinese, Spanish and Americane were evaluated to infect with *Pyrenochaeta terrestris* to study their resistance and susceptibility. The results indicated that Balady cv.was the most resistant one, while, Chinese and Seds-40 were the most susceptible cvs. The levels of histones, peroxidase and phenoloxidase activity, as well as RNA content were higher in inoculated leaves of resistant garlic cultivar (Balady) than susceptible one (Seds-40).Total chlorophyll of garlic had not any significant importance in the susceptibility to pink root disease.

Key words: Garlic cultivars, Biochemical changes, *Pyrenochaeta terrestris.*

INTRODUCTION

Pink root is one of the most important diseases which attacked cultivated *Allium* spp. (Anonymous,1973). Pink root caused by *Pyrenochaeta terrestris*, has been reported as a serious disease to garlic in Egypt by Shalaby *et al.*, (2002).

Ahmed *et al.*,(1991) and Tadrous (1991) indicated that oxidative enzymes activity (peroxidase and phenoloxidase) were correlated negatively with the susceptibility of the onion cultivars to infect with *P.terrestris*. Zappacosta , *et al.*,(2003) reported that peroxidase activity was enhanced in root of onion grown in *Phoma terrestris* inoculated soil compared to healthy control plants.

Heitefuss and Wolf (1976) stated that most work on nuclic metabolism in host interaction has been done with the obligately

biotrophic parasites , *Puccinia* spp. *Erysiphe* spp. and *Plasmodiophare* spp. with a few studies on *Phytophthora* spp and *Fusarium* spp.

Chlorosis is one of the most common symptoms of plant diseases and is indication of a reduction in the chlorophyll content of green tissues (Agrios, 1988). John (1998) indicated that chlorosis associated with some virus infection., higher levels of chlorophyllase enzyme have been dected, suggesting that chlorophyll is being degraded by the enzymatic reaction.

The purpose of the present study was develop biological assays to evaluate disease reaction with garlic cultivars when inoculated with *Pyrenochaeta terrestris.*

MATERIALS AND METHODS

Pathogenic isolate of the *P. terrestris* was isolated from an infected garlic fields in EL-Ismailia governorate. The fungus was identified as mentioned by Barnett (1960). The isolated fungus was grown on Czapek's medium for 30 days. The mycelial and broth were blended for 30 sec at low speed in a warring Blender, then 200 ml inoculum and 1.400 ml of distilled water were mixed with 23.5 kg of sterile soil (Rengwalska and Simon, 1986) and used as inoculum . The pathogenicity tests of the isolated fungus was done in the greenhouse.

Cultivars Susceptibility

Five cultivars (Balady, Seds-40, Chinese, Spanish and Americane),were tested for their susceptibility to *P.terrestris* and they were obtained from the Agric. Center, Giza, Egypt. The obtained cultivars were sown in infested soil in pots (30 cm in diam),at the rate of two cloves / pot. Every cultivar was represented by 10 pots. Disease development was observed 90 days after inoculation and the disease incidence was determined, 100 days after planting.

Biochemical Analysis

Garlic plants, Balady (resistant) and seds-40 (susceptible) were used in this study. Garlic cloves were sown in the infested pots as previously mentioned, and 20 pots were uninoculated to serve as control. Three samples were taken after 100,110 and 120 days after planting and named sample 1,2 and 3, respectively. Every sample was

represented at random choice from four pots. Higher molecular weight basic protein (histones poly lysine and poly arginine) was estimated according to Johns (1964) and Bonner, *et al.*,(1968). Peroxidase and phenoloxidase enzymes activity were estimated according to the methods given by Allam and Hollis (1972) as well as Matta and Dimond (1963), respectively. RNA content was estimated by the method described by Nitson and Lang (1966) and free phenols were determined as mentioned by Anonymous (1960).

RESULTIS AND DISCUSSION

There is a great difference between investigated cultivars in their susceptibility to the pink root disease Table 1. Balady cultivar showed less degree of infection with significant differences with other cultivars. On the other hand, cultivars Spanish, Seds-40; Chinese and Americane showed a great tendency to infection. Variation in variatal reaction of garlic cultivars to the pink root disease was stated by numorous investigators under greenhouse conditions such as Rengwalska and Simon (1986); Tadrous (1991); Pages and Midmore (1994); Thornton and Mohan (1996) and Colemen, *et al.*,(1997).

Generally, Balady cultivar was less infection with pink root disease. On the other contrarily, Seds-40 was highly susceptible.

The differences in cultivars susceptibility to infect with *P. terrestris* might be due to the variation in genetic make up for each cultivar (Walker,1975). On the other hand, the differences in cultivars resistant might be due to the content of histones in the plant tissues(Hadwiger *et al.*, 1977).

Table 1. Susceptibility of garlic cultivars to the pink root disease caused by *P. terrestris*

Garlic cultivars	Disease incidence%, 100 days after planting
Balady*	02.00
Seds-40*	69.30
Chinese*	70.10
Spanish*	25.70
Americane*	58.10
L.S. D. 5%	4.97

*control values of these cultivars were considered to be 0%

The leaves of cultivar Balady (resistant) was characterized by their significant higher content of histones (polylysine and polyarginine) , 100 days after planting and whether the plants were healthy or infected, in comparison with Seds-40 ones, The results obtained were in line with those of Hadwiger, *et al.*, (1977) who cleared that basis proteins rich in lysine and arginine are potentially more important in the pea tissues resistance to plant pathogenic fungi . These histones inhibit the growth of *F. solani* f. sp. *phaseoli in vitro*.

Ruiz – Carrillo , *et al.*,(1975) indicated that histones are synthesized in the cytoplasme and transported to the nucleus in synchronization with DNA synthesis within the cell. Once they have become localized in nucleoprotein they might be unavailable to the fungus. However, Hadwiger *et al.*,(1977) cleared that changes in arrangement , or number or potential activation of histones genes could influence the potential for activation of histones genes could influence the potential for disease resistance.

It might be concluded that the resistant cultivar (Balady) contained higher amount of histones and therefore the plant can resist the *P.terrestris*. In other words, there is a positive correlation between histones content and resistance of garlic to *P.terrestris*.

Table 2. Effect of pink root disease on histones content of resistance and susceptible garlic leave

Garlic cultivars		Histanes*(ug/gm fresh weight).		
		Ploylysine	**Polyarginine**	**Total**
Balady	**healthy**	11.13	10.97	22
(resistant)	**infected**	13.12	18.88	32
Seds-40	**healthy**	10.10	9.90	20
(susceptible)	**infected**	11.00	10.00	21
L.S.D. 5 %		1.39	1.68	3.15

*These compound were estimated, 100 days after planting.

Data in Table 3 indicate that, the infection by *P.terrestris* significantly increased the activity of both peroxidase and phenoloxidase enzymes in the leaves of resistance cultivar (Balady) in sample 1,2 and 3,as compared with the healthy one. While the opposite was detected of both enzymes in the susceptible cultivar (Seds-40). It is also clear that, increased the periods of taking samples lead to increasing the exidative enzymes in both healthy and infected cultivars Table 3.

The raised peroxidase and phenoloxidase activaties which occurred to *P.terrestris* are in concord with the result published by Ahmed *et al.*,(1991) who reported that activities of peroxidase and phenoloxidase increased in leaves of resistant garlic plants after infection by *P. terrestris*.

The infection with *P. terrestris* results in significant increased of RNA content in the leaves of resistant plant (Balady) in all sampled periods, as compared to the healthy one, Table 4. The leaves of susceptible plant (Seds-40) were characterized by their low content of RNA and whether the plants healthy or infected.

Such finding might indicated that RNA play a role in resistance and susceptibility of garlic plant, to *P. terretris* via their physiological role in different metabolic pathways.

Shalaby and Saeed (2000) indicated that, increased resistance of sesame plants against wilt disease might be due to RNA content which played a great roll in a biochemical mechanism for induced systemic resistance.

Table 3. Effect of pink root Disease on oxidative enzymes activity of garlic leaves (as optical density of min)

Garlic cultivars		Peroxidase			Phenoloxidase		
		Sample1*	Sample2*	Sample3*	Sample1*	Sample2*	Sample 3*
Balady	**healthy**	8.70	18.67	41.30	6.58	13.37	27.07
(resistant)	**infected**	10.42	22.77	54.37	8.28	16.53	30.07
Seds-40	**healthy**	8.13	22.80	45.95	9.62	16.83	28.50
(susceptible)	**infected**	7.73	20.83	40.13	8.83	13.13	20.37
L. S. D. 5%		1.13	1.22	1.88	1.82	1.99	2.01

Sample 1: 100 days after planting. Sample 2: 110 days after planting.
Sample 3: 120 days after planting.

We can infer from the data present in Table 5 that, the infection by *P.terrestris* resulted in decrements in total chlorophyll content in the leaves of both Balady and Seds-40 garlic cultivars. This decrement were distinct and significant in infected Seds-40 garlic cultivar by the pathogen in all tested samples, Table 5.

The decrement in chlorophyll content in infected leaves of Balady and Seds-40 cultivars was also reported in other crops by Shalaby and El-Korashy (1996) and Shalaby *et al.*,(1997).

The adverse effect of the fungus *(P. terrestris)* on chlorophyll might be due to the breakdown of chlorophyll, inhibition of chlorophyll synthesis, and / or a reduction in the number of chloroplasts (John, 1998).

The decline noticed in chlorophyll as a result of the

Table 4. Effect of pink root disease on RNA content of garlic leaves

Garlic cultivars		RNA*(ug/g, fresh weight).		
		Sample 1	Sample 2	Sample 3
Balady	**healthy**	22.60	53.50	41.70
(resistant)	**infected**	26.10	64.80	49.70
Seds-40	**healthy**	22.60	19.10	40.50
(susceptible)	**infected**	22.10	19.30	42.10
L. S. D. 5%		2.71	4.31	4.01

*RNA: Ribonucleic acid.

Table 5. Effect of pink root disease on total chlorophyll of garlic leaves

Garlic cultivars		Total chlorophyll(mg/g. fresh weight)		
		Sample 1	Sample 2	Sample 3
Balady	**healthy**	1.23	2.89	3.28
(resistant)	**infected**	1.21	2.86	3.19
Seds-40	**healthy**	1.17	3.71	4.01
(susceptible)	**infected**	0.84	2.03	1.98
L. S. D. 5%		0.11	0.72	0.88

infection might also attributed to the deter of water absorption due to root decay (Sterne *et al.*, 1978) Hence drought inhibit the activity

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of some enzyme which may cause and increase in the rate of chlorophyll biosynthesis (Kamel *et al.*,1995).

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تقييم بعض أصناف الثوم للإصابة بمرض الجذر القرنفلي المتسبب عند الفطر بيرينوكيتا ترستس وعلاقتها ببعض التغيرات البيوكيميائية

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تم تقييم القابلية للإصابة والمقاومة لأصناف الثوم البلدي، وسدس ٤٠ والصيني والأسباني والأمريكاني للإصابة بمرض الجذر القرنفلي. وقد تبين أن الصنف البلدي كان أكثر الأصناف مقاومة للإصابة المرضية بينما كان الصنف الصيني وسدس ٤٠ أشد الأصناف قابلية للإصابة المرضية. وقد تميز الصنف المقاوم (البلدي) المعدي بالفطر بيرينوكيتا ترستس بزيادة محتوي أوراقة من الهستوتات ونشاط إنزيم البيروكيسيديز والفينول أوكسيديز والحمض النووي ريبوينوكليك وذلك بالمقارنة بالصنف القابل للإصابة المعدي(سدس ٤٠).لم يتضح أن للكلوروفيل أي دور في حساسية ومقاومة أصناف الثوم المختبرة للإصابة بمرض الجذر القرنفلي.