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THE ROLE OF HYPHAL CONTENTS OF AMINO ACIDS IN VIRULENCE OF *Rhizoctonia solani* (THE INCITANT OF BEAN ROOT ROT).

Darweish, Aziza.K.
Plant Pathology Department, Alexandria University. Egypt.

ABSTRACT

The quantitative and qualitative contents of free and total amino acids were estimated in hyphae of six isolates of *Rhizoctonia solani.* Such isolates were varied in their virulence on bean (*Phasiolus vulgaris*) Giza-3 cultivar. It has been found that the mycelium of the high virulent isolates (4,5,6) contained higher amounts of total amino acids than that of the less virulent isolates (1,2,3). The mean content of lucine was the highest 1760.76 mg/100g fresh weight of the total amino acids of all fungal isolates. However, the least means of total amino acids were found with proline 107.352 mg/100g f.w and cystine 16.710. The rest of total amino acids were varied between the fungal isolates in which the high virulent isolates (4,5,6) contained high amount of Glutamic (1086.164, 1245.230 and 2202.330 mg/100g f.w respectively), Glycine (1346.484, 1431.663 and 2530.702 mg/100g f.w respectively) and lucine (2009.396, 2194.982 and 3465.961 mg/100g f.w respectively). On the other hand Histidine was found also in high concentration (755.084 mg/100g f.w) in hyphae of the high virulent isolate No.6 which could be related to virulenc.

In case of free amino acid, the highest contents means were found with thrionine (105.442 mg/100g f.w) and glutamic (144.803 mg/100g f.w). Both high virulent isolates 5 and 6 contained the high amounts of Aspartic (64.810 and 136.821 mg/100g f.w respectively) and Glutamic (218.626 and 294.383 mg/100g f.w respectively) . The least amounts of free amino acids in hyphae of all isolates were proline, Cystine, Lucine and Phenylalanine. The distribution of free amino acids concentrations was varied between the different isolats and does not follow specific trend. Such data indicate that virulence of certain fungal isolates could be related to the amount of some total amino acids rather than free amino acids.

Keywords: root-rot, damping-off, common bean, amino acid, *Phaseolus vulgaris, Rhizoctonia solani,*

INTRODUCTION

Bean (Phaseolus vulgaris L.) is one of the most important leguminous crops in Egypt and in the world. Green pods as well as seeds are used for fresh meal . Bean generally could be attacked by many soil-borne fungi which cause damping-off and root-rot diseases. *Rhizoctonia solani* (Kuhn) is reported to be one of the main pathogens causing root rot disease as well as damping-off of bean and other crops in Egypt and all over the world (El-Helaly *et al.,* 1966, Hoch and Hagedorn 1975, Darweish 1978, Mabrouk 1984, Abdel-Rehim *et al.,* 1992,1994, Abdel- Kader 1997).

The present study was carried out to investigate the role of hyphal contents of total and free amino acids in the virulence of six isolates of *Rhizoctonia solani.*

MATERIALS AND METHODS

Samples of bean plants showing root rot symptoms, collected from bean grown in the Alexandria University, Experiment Station and also from several fields in Kafer El-Sheikh. The infected seedlings were washed thoroughly with tap water, and then were surface sterilized by dipping in ethyl alcohol then flamed. The sterilized plant materials were plated on potato dextrose agar and Czapeck-dox agar media. The purified fungal isolates were identified as six isolates of *Rhizoctonia solani* based on cultural and microscopical examinations according to Gillman (1957) and Barnett &Hunter, (1972). Virulence of *R. solani* isolates on bean was tested under greenhouse conditions. Clay pots (15 cm diameter) containing sterilized sandy loam soil were artificially infested individually with the inoculum of each tested isolate which grown in 100 ml conical flasks, containing 30 ml of potato dextrose liquid medium for two weeks at 25 $\pm$ 1°C. Fungal growth developed in each flask was filtered and blended with 100 ml tap water in a warring blender. Inocula suspensions were applied at rate of 50 ml per pot. Four pots were used as replicates for each treatment as well as control (uninfested soil). Disinfected bean seeds cv Giza 3, were sown at the rate of 5 seeds/pot. Fungal infection on bean was observed throughout the growth period and the percentage of pre and post-emergence damping-off was recorded.

Preparation of mycelial extracts :

Equal disks of young growing mycelium (5mm in diameter) from the edges of each isolate of *Rhizoctonia solani* were transferred into 250 ml conical flasks, each containing 100 ml Czapek-Dox liquid medium, then were incubted at 25 $\pm$ 1°C for 15 days. Mycelial growth of each isolate was collected, filtered through Watman's No.1 filter paper and washed twice with sterilized distilled water, then used for amino acids extraction.

Total amino acids extraction:

Extracts of amino acids were prepared according the method of Moore *et al.,* (1958); in which one gram fresh weight of each sample was mixed with 10ml of 6 N hydrochloric acid (HCl) using digestion tube that was then sealed under vacuum. Contents of each sealed tube were digested for 24 hours at 105 °C, cooled and filtered then washed with distilled water. The filtrate was dried at room temperature till a powdery dry film was obtained, then redissolved in 5 ml citrate buffer solution 2.2 Normal. Twenty microliters of each prepared filtrate were then injected in the instrument capsule of Beckman 116 GI amino acid analyzer apparatus.

Free amino acids extraction:

Extracts of free amino acids were prepared according to Hamilton's method (1962). The mixture was centrifuged for 5 minutes at 3000 r.p.m. and the supernatant was filtered through Watman's No.1 filter paper. Filtrates

were then diluted with 1:1 (v:v)of 2.2 normal citrate buffer solution (Mondino *et al.,* 1972), then injected in Beckman 116 GI amino acids analyzer apparatus.

Standard mixture of the common 17 amino acids was prepared and treated as fungal extracts, then injected into the amino acid analyzer to be used for qualitative and quantitative determination.

All amino acids were expressed as milligrams amino acids /100 gram fresh weight of mycelial samples.

RESULTS AND DISCUSSION

Greenhouse pathogenicity experiment revealed that the six tested isolates of *R. solani* were pathogenic to bean (*Phaseolus vulgaris* L.) Giza3 cultivar as recorded in Table (1). They could attack germinated seeds, seedlings as well as bean plants causing different sizes of lesions . Even that some variations were observed between fungal isolates in their virulence in which isolates (4,5,6) were more virulent than isolates (1,2,3) in total damping-off. However, there were no significant differences between most isolates in their effect on post-and pre-emergence damping –off.

These results confirmed those recorded by many other investigators (Mabrouk, 1984, Fahim 1987, Abdel-Kader 1997).

Table (1): The percentage of damping –off of bean plants infected with different isolates of *R. solani*

Fungal isolate	% Damping-off			survivals
	Pre-emergence	Post-emergence	Total	
1	25	15	40	60
2	20	30	50	50
3	35	25	55	45
4	40	20	60	40
5	30	35	65	35
6	35	40	75	25
Control	20	0.0	20	80
L.S.D $_{0.05}$	22.26	23.92	18.92	20.14

Data presented in Table (2) and Fig (1) indicate that the mycelia of the most virulent isolates of *R. solani* (4,5,6) were significantly containing more total amino acids than the least virulent isolates (1,2,3) . In general, proline and cystine were significantly the least amount of amino acids than the rest of total amino acids. Significant variations were also detected in the amount of total amino acids within each isolate. Even the Leucine mean amount of the total amino acids was the highest in all fungal isolates (1760.76 mg/100g f.w) but still some variations in Leucine contents were noticed between the individual fungal isolates. The high amount of Aspartic, Glutamic, glycine, Alanine, leucine, and lycine in all virulent isolates (4,5,6) might be recommend as indicators for virulence in which the more concentration of them in hyphal extraction the more virulent isolate. Histidine was found in higher concentration (755.084 mg/100 g f.w) in the most virulent isolate (Isol. No.6)in which might be an extra total amino acid related to virulence.

Data present in Table (3) and Fig (2) indicated that the extracted free amino acids from fungal hyphae of the most virulent isolates (5,6) were significantly higher in case of Aspartic (64.810,136.821 mg/100 g f.w respectively) and Glutamic (218.626, 294.383 mg/100 g f.w respectively). However, the Proline, Cystine, Isoleucine, Leucine and Phenylalanine were found in lower amounts in almost all isolates than the rest of amino acids . Even the means of both Threonine and Glutamic acid were the highest in quantity (105,442 and 144.803 mg/100 g f.w respectively) than the other free amino acids, but the actual concentration of each isolate does not follow the virulence trend, in which the less virulent isolate No.3 had higher concentration of Glutamic (105.856 mg/100 g f.w) than the higher virulent isolate No.4 (91.814 mg/100 g f.w.). The less virulent isolate No. 2 contained higher concentration of Threonine (150.503 mg/100g f.w) than the most virulent isolate No.6 (24.484 mg/100 g f.w).

Concentrations of the rest of free amino acids does not follow a noticable trend related to virulence. Concentrations of the free amino acids in general were far less than that of total amino acids.

Even the six isolates of fungi could be descendingly ranked 6,5,4,3,2,1 according to theirs pathogenicity to match their contents of total amino acids, but not to match with their contents of free amino acids.

So it seems that virulence of *R. solani* should be correlated to the total amount of amino acids rather than free amino acids within hyphal cells. Such results are in agreement with previous finding of Abou-Elseoud *et al.,* (1990) who reported that in a comparative analysis of protein patterns of some *Cephalosporium maydis* isolates revealed that virulence was associated with some specific protein bands which were absent in avirulent isolates. Saeed *et al.,* (1994) found that mycelia of two isolates of *Trichoderma harzianum* which were tolerant to the fungicides Ronilan and Sumisclex were containing higher amount of total and free amino acids than the non-tolerant isolates.

Table (2) : Total amino acids content in the mycelium of six isolates of *Rhizoctania solani* (mg/100 g f.w.)

Amino Acid		Isolates						Mean	L.S.D 0.05
		1	2	3	4	5	6		
Aspartic Acid	(As.)	272.558	347.313	362.891	725.030	736.233	907.752	**558.63**	**4.79**
Threonine	(Th.)	192.029	259.804	227.228	408.778	460.453	627.174	**362.578**	**3.04**
Serine	(Se.)	373.868	302.707	269.307	440.196	639.499	888.497	**485.679**	**4.31**
Glutamic Acid	(Gt.)	466.858	1010.274	557.466	1086.164	1245.230	2202.330	**1094.72**	**11.33**
Proline	(Pr.)	058.848	76.613	063.623	101.504	153.719	189.803	**107.352**	**0.97**
Glycine	(GL.)	524.467	992.568	703.565	1346.484	1431.663	2530.702	**1254.91**	**0.27**
Alanine	(Al.)	340.697	426.821	370.297	708.458	841.409	1058.356	**624.34**	**5.32**
Cystine	(Cy.)	7.123	6.810	13.465	3.465	28.140	41.261	**16.7107**	**0.27**
Valine	(Va.)	154.862	274.105	227.564	401.159	448.494	722.075	**371.377**	**3.70**
Methionine	(Me.)	71.237	84.275	67.327	114.969	208.945	316.338	**172.099**	**1.81**
Isoleucine	(Is.)	109.023	210.431	183.802	298.298	320.101	487.229	**268.147**	**2.41**
Leucine	(Le.)	722.690	1266.673	904.873	2009.369	2194.982	3465.961	**1760.76**	**18.59**
Tyrosine	(Ty.)	142.473	190.682	188.515	326.608	422.112	483.790	**292.363**	**2.55**
Phenylalanine	(Ph.)	158.579	221.668	232.277	397.040	474.876	730.671	**369.185**	**3.88**
Histidine	(Hi.)	185.834	324.840	181.782	379.087	405.227	755.084	**371.976**	**3.82**
Lysine	(Ly.)	274.209	455.253	367.604	527.890	777.389	1275.666	**613.002**	**6.68**
Arginine	(Ar.)	156.101	252.313	218.139	353.883	438.644	596.915	**335.999**	**2.96**
Mean		**247.733**	**394.303**	**312.307**	**566.3754**	**660.419**	**1016.4473**		

Table (3) : Free amino acids content in the my celium of Six isolates of *Rhizoctania solami* (mg/100 g f.w.)

Amino Acid		Isolates						Mean	L.S.D 0.05
		1	2	3	4	5	6		
Aspartic Acid	(As.)	30.3	20.307	51.408	38.022	64.810	136.821	**56.8997**	**0.77**
Threonine	(Th.)	41.190	150.503	19.983	254.488	142.006	24.484	**105.442**	**1.70**
Serine	(Se.)	17.786	63.369	9.721	123.139	57.537	28.516	**50.0113**	**0.76**
Glutamic Acid	(Gt.)	91.383	66.754	105.856	91.814	218.626	294.383	**144.803**	**1.65**
Proline	(Pr.)	5.040	6.120	2.160	4.321	8.641	5.760	**5.34033**	**0.041**
Glycine	(GL.)	15.554	36.941	8.749	97.215	75.611	42.342	**46.0687**	**0.63**
Alanine	(Al.)	25.203	39.293	28.804	193.782	168.506	43.207	**83.1325**	**1.40**
Cystine	(Cy.)	0.900	2.700	1.080	17.643	6.481	3.600	**5.40067**	**0.12**
Valine	(Va.)	4.50	8.929	10.081	31.325	29.457	4.321	**14.7688**	**0.22**
Methionine	(Me.)	11.017	19.011	16.238	139.341	32.261	122.059	**56.6545**	**1.06**
Isoleucine	(Is.)	2.880	2.340	0.720	10.8017	23.188	4.752	**7.44695**	**0.154**
Leucine	(Le.)	5.040	4.897	2.484	16.131	21.531	3.168	**8.87517**	**0.14**
Tyrosine	(Ty.)	3.600	8.281	4.680	31.685	11.161	9.361	**11.4613**	**0.19**
Phenylalanine	(Ph.)	3.240	3.312	2.916	7.201	3.241	4.321	**4.0385**	**0.03**
Histidine	(Hi.)	9.073	26.572	11.018	85.549	32.405	45.367	**34.9973**	**0.51**
Lysine	(Ly.)	6.481	18.002	9.794	64.810	11.522	8.641	**19.875**	**0.41**
Arginine	(Ar.)	3.348	11.665	7.993	40.830	15.122	10.802	**14.96**	**0.24**
Mean		**16.2509**	**28.7645**	**17.2756**	**73.4175**	**54.2415**	**46.5826**		

Fig

Fig

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دور الأحماض الأمينية بهيفات فطر ريزوكتونيا سولانى (المسبب لعفن جذور الفاصوليا) على قدرته المرضية

عزيزة كامل درويش
قسم أمراض النبات – كلية الزراعة (الشاطبى) – جامعة الإسكندرية – مصر.

أظهرت التقديرات الكمية والنوعية للمحتويات الخلوية من الأحماض الأمينية الحرة والكلية مقدرة بملليجرام/100جم وزن رطب من الميسيلوم بهيفات ستة عزلات من الفطر ريزوكتونيا سولانى *Rhizoctonia solani* والتى تم إختبار القدرة المرضية لها على محصول الفاصوليا صنف جيزة 3 ، أن ميسيلوم العزلات الفطرية (4و5و6) ذات القدرة المرضية العالية تحتوى على كمية مرتفعة من الأحماض الأمينية الكلية عنها فى العزلات ذات القدرة المرضية الأقل (1و2و3). وكان متوسط كمية كل من الأحماض الأمينية بروالين 107,352 وسيستين 16.7107 مليجرام/100جرام وزن رطب من الميسلوم أقل من باقى الأحماض الأمينية الكلية فى جميع العزلات . وكان متوسط كمية الحمض الأمينى ليوسين 1760.76 يمثل أعلى متوسط لكمية الأحماض الأمينية الكلية بصفة عامة . أما باقى الأحماض الأمينية الكلية فقد أختلفت كمياتها المقدرة للعزلات المختلفة حسب قدرتها المرضية حيث تميزت العزلات الفطرية 4و5و6 ذات القدرة المرضية العالية بإحتوائها على كمية عالية من كل من الأحماض الأمينية الكلية كالآتى :
جلوتاميك (108.164) ، (1245.230) ، (2202.330) على التوالى. والجليسين (1346.384) ، (1431.663) ، (2530.702) على التوالى . وليوسين (2009.369) ، (2194.982) ، (3465.961) على التوالى. وجد أن الحمض الأمينى هستيدين يوجد بأعلى كمية (755.084 مجم/100جم وزن رطب) فى العزلة رقم 6 ذات القدرة المرضية العالية عن باقى العزلات مما يدل على إحتمال وجود علاقة بين محتوى الميسيلوم لهذا الحمض وزيادة القدرة المرضية له.
اما بالنسبة للأحماض الأمينية الحرة وجد ان المتوسط العام لكمية الأحماض الأمينية كان أعلى ما يمكن فى حالة ثريونين (105.442) وجلوتاميك (144.803) . وكانت كل من العزلتين 5و6 ذات القدرة المرضية العالية محتوية على أعلى كمية من أسبارتك (64.810) (136.821) على التوالى وجلوتاميك (218.626) (294.383) على التوالى عن الموجود فى باقى العزلات.
كما وجد أيضا أن الأحماض الأمينية الحرة بروالين وسيستين وايزوليوسين وليوسين وفينيل ألانين توجد بكمية ضئيلة عن الأحماض الأخرى فى جميع العزلاتز وجد أيضا أن متوسط المحتوى العام للأحماض الامينية الحرة بكل من العزلات العالية القدرة المرضية (4و5و6) كان 73.4157 و 54.2415 و64.5826 مجم/100جم وزن رطب على التوالى كان أكبر مما وجد بالعزلات الأقل فى القدرة المرضية (1و2و3) هى 16.2509 و 28.7645 و 17.2756 مجم/100جم وزن رطب على التوالى ألا أن توزيع كميات الأحماض الأمينية الحرة كل على حدة للعزلات الفطرية المختلفة كان متباينا وغير مرتبط بقدرتها المرضية .
وبالتالى أظهرت النتائج أنه يمكن ربط القدرة المرضية للعزلات المختلفة بمحتواها من الأحماض الأمينية الكلية وليس بمحتواها من الأحماض الأمينية الحرة.