Efficiency of Different Biocontol Agents on both Susceptible and Resistant Bean Plants and their Protein Pattern Consequences

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Abstract: Five *Streptomyces* Spp. namely *St. albadncus, St. vastus, St. griseoplanus, St. murinus* and *St. lydicus* were screened for their efficiency to control *Rhizoctonia solani* root rot pathogen *in vitro*. Results proved that *Streptomyces lydicus* was the most potent biocontrol agents against the fungal pathogen tested. However, the experiment was conducted to a greenhouse to investigate the differences in protein pattern between resistant and susceptible varieties of bean plants in response to biological control to investigate the mechanism of pathogen related protein in pathogenicity. Results *in vivo* showed that the biocotol used obviously reduced the infection percentage up on susceptible bean variety down to 94/22 and for resistant variety to 39/6. Accordingly, the growth parameters also revealed that the response of the susceptible plants were generally more than that of the resistant one. Interestingly, results of protein pattern clarify that the highest protein bands as well as the unique bands were only detected in both susceptible control and resistant infected bean plants treated with the biocontol agent respectively. Furthermore, the genetic distance (GD) results revealed that the highest GD was detected also between the two mentioned treatments. In addition, the data obtained from the genetic similarity of protein pattern proved that the lowest similarity was also between both the susceptible control and resistant infected bean plants treated with biocontol agent respectively. Amazingly, the highest genetic similarity of protein pattern was detected between both susceptible infected bean plants treated with biocontrol and resistant control one. Finally, our results suggested that there are a great similarity between the susceptible infected variety treated with biocontrol agent and the resistant control untreated variety but not between the resistant infected variety treated with biocontrol agent and the susceptible control untreated variety. This may also give an impression that the pathogen resistant protein (PR) works independently in the susceptible plants but works dependently in the resistant one.
[Ayman A. Farrag. Efficiency of Different Biocontol Agents on both Susceptible and Resistant Bean Plants and their Protein Pattern Consequences. Journal of American Science 2011;7(4):7-14]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Biological control; *Streptomyces Spp.*; *Phaseolus vulgaris*; Electrophoresis protein pattern

1. Introduction

Root rot of common bean (*Phaseolus vulgaris* L.) is a soil-borne disease that is incited by several fungal pathogens including *Fusarium* spp., *Pythium* spp. and *Rhizoctonia solani*. It occurs in all bean-growing areas of the world leading to enormous crop losses. The pathogen is known to be very persistent in soil and capable of surviving in infested fields for very long period and is difficult to control (Burke and Hall, 1991). Disease management options include crop rotation, improving soil fertility levels, use of resistant cultivars, use of fungicides and biological control, but, the impetus for developing biological control agents has been the public perception of pesticide toxicity in the environment (Saman, 2007). Moreover, Mahade Van and Crawford (1997) identified *Streptomyces lydicus* was as abroad spectrum biocontrol agent that proceed to produce an extracellular chitinase enzyme and, one or more antifungal agents. In spite of, changes in amino acid pattern of plants as affected by fungal infection have received adequate attention as reported by Benedict and Hildebrand (1958).

Our knowledge of molecular events occurring during plant pathogen interactions has expanded significantly in the last ten years. Based on this knowledge, several strategies have emerged for developing crop varieties resistant to pathogens. Strategies include the manipulation of resistance by expression of PR proteins. In these cases the observed resistance was not absolute and was restricted to a resistance to limited number of fungi (Grover and Pental, 2003). However, Mauch-Mani and Metraux, 1998), also mentioned that, activation of disease resistance response in a host plant frequently requires the interaction of a plant gene product with corresponding pathogen derived signal and the products of the resistance genes from diverse plant species show a remarkable structural similarity.

Finally, the objective of this study aims at understanding more about the mechanism of pathogen related proteins (PR), in controlling fungal pathogenicity, and its role played in plant immune system.

2. Material and Methods

Pathogen and biocontrol agents

Rhizoctonia solani f. sp (*Phaseoli*) was isolated from diseased common bean plant root tissues and maintained on Dox agar medium and then identified at the Agriculture Research Center. Also, the identified *Streptomyces* strains; *St. murinus, St. griseoplanus, St. vastus, St. albadncus* and *St. lydicus* used in this study as a biocontrol agent was kindly Provided**.**

Plant material

A pure strain of both susceptible variety (Red Mexico) and resistant variety (Prince) of common bean plant (*Phaseolus vulgaris* L.) was kindly provided by the Agriculture Research Center (A.R.C.), Giza, Egypt.

Antimicrobial activity test

The antimicrobial activities of the five tested *Streptomyces* spp., *St. murinus, St. griseoplanus, St. vastus, St. albadncus* and *St. lydicus* against root rot bean pathogen *Rhizoctonia solani* were assed using the cup plate assay method described by Kavanagh (1972).

Plant cultivation and inoculation

According to Fuchs and Sacristan (1996), a six seeds were cultivated in 5 cm in diameter pots containing 2 Kg of natural (non sterilized) sandy clay soil. Two sets of pots were prepared, the first as susceptible and the second as resistant plants were used in triplicates. Accordingly, the following treatments were prepared for each set as follow:

- uninoculated bean plants (control).
- Bean plants inoculated with the biocontrol *Streptomyces lydicus***.**
- Bean plants inoculated with the root rot pathogen *Rhizoctonia solani*.
- Bean plants inoculated with both root rot pathogen and the biocontrol agent as mentioned above.

Phytopathological analysis

Disease symptoms were assessed, 30 days after inoculation and the disease index was evaluated for severity of root rot and foliar symptoms according to Leath *et al.* (1989) by using a scale consisting of five classes, 0 (no symptoms), 1 (slight and few small lesion on the main tap root), 2 (yellowing and moderate lesion cover up the main tap root), 3 (plant wilted and the root system affected) and 4 (plant severely stunted and the root system was completely destroyed)

Disease Index (DI) was calculated using the five grade scale, according to the formula:

DI = (1n 1 + 2n 2 + 3n 3 + 4n 4) 100 / 4Nt.

Where n 1 to n 4 is the number of plants in the indicated classes, and, Nt is the total number of plants tested.

Determination of chlorophyll content

Chlorophyll content was determined according to the method of Vernon and Seely (1966). The pigment was extracted by grinding 1 g of fresh leaves with a suitable amount of 100 ml of 80% aqueous acetone (v/v). The optical density of the extract was measured using Carl Zeiss Colorimeter at two wave lengths (649 and 665 nm) the pigment content was calculated using the equation of this method and expressed as mg/g fresh weight.

Electrophoretic analysis of protein by SDS-PAGE

SDS-PAGE was used for detection of genetic variability among resistant and susceptible plants (*Phaseolus vulgaris* L.) for the determination of quantitative and qualitative of the tris and tris/SDS-soluble proteins. This method was done according to Laemmli (1970) as modified by Studier (1973). In this protocol, electrophoresis is in a vertical slab gel between glass plates. The gel consists of two parts, the upper stacking gel (5%) and the lower resolving gel (15%).

Gel analysis

The gel analysis was applied by AlphaEaseFC^{TM} ver. 4 software. The characters and states have been subjected to the numerical analysis under Multi Variate Statistical Analysis (MVSP) ver. 3.13p software using similarity and dissimilarity assessment percentage method. The method applied is based on cluster analysis by using an UPGMA, dendrogram illustrating the interspecific relationships of studied samples as percent similarity.

Also, genetic distance was calculated between the sasmples through MVSP ver. 3.13p software.

3. Results and Discussion

The antagonistic activity of different *Streptomyces* spp. tested against the pathogenic fungus *Rhizoctonia solani in vetro*

Results of the antagonistic ability of the five *Streptomyces* species tested against *Rhizoctonia solani* as plant pathogenic fungus by applying the cup plate method. Table (1) revealed that, there are only one *Streptomyces* sp. namely *St. lydicus* exhibited highly antagonistic potency against the pathogenic fungus *Rhizoctonia solani.* Any way the rest *Streptomyces spp.* tested; namely *St. albadncus, St. vastus, St. griseoplanus* and *St. murinus* exhibited less antagonistic activity against the root rot Pathogen. Fortunately, the obtained results are parallel to that

obtained by many authors as, Ibrahim *et al.* (2001) and Rasmy (2002), as they proved that most species of the biocontrol agent are able to antagonize many plant pathogenic fungi and sometimes give an equal control effects to those obtained by certain fungicides.

Table 1. The antagonistic activity of different *Streptomyces* spp. tested against the pathogenic fungus *Rhizoctonia solani in vetro*

Types of *Streptomyces* tested	Mean values of inhibition zones / mm
1. *Streptomyces murinus*	20
2. *Streptomyces griseoplanus*	23
3. *Streptomyces vastus*	25
4. *Streptomyces albadncus*	26
5. *Streptomyces lydicus*	27

A greenhouse biological control of *Rhizoctonia solani* root rot disease on *Phaseolus vulgaris* susceptible and resistant plants by *Streptomyces lydicus*

Nearly all fields and vegetable crops are suffering from at least one or more fungal plant pathogens and considerable yield losses were recorded which sometimes exceeds 70% (Watkins, 1981 and Morris *et al*., 1984). Also, microscopic studies of the infection process to different plant hosts by fungal pathogenic isolate of *Rhizoctonia solani* have been well documented by Stockwell and Hanchey (1983).

Any way, the results obtained from table (2) revealed that, the plant pathogenic fungus *Rhizoctonia solani* exerts a drastic effect on the roots of both susceptible and resistant bean plant variety. However, the disease development in susceptible variety was more deleterious than in resistant one. The results obtained are in consistent with that of Burke and Miller (1983) as they proved that root rot pathogen can almost destroy a bean crop, even at highest level of resistance to the disease.

Results also showed that, the use of *Streptomyces lydicus* as a biocontrol agent was able to minimize the drastic action of the pathogenic fungus *Rhizoctonia solani* upon both the susceptible and resistant bean plant varieties by reducing the infection percentage from (94 & 39%) down to only (22 & 6%) for both susceptible and resistant bean plant variety respectively. Interestingly, these results are in agree with that of Mahadevan and Crawford (1997) as they found that, *Streptomyces lydicus* was identified as abroad spectrum as a biocontrol agent that proceed to produce an extracellular chitinase enzyme, beside one or more of antifungal agents. Any way, all of the other treatments displayed different degree of control values, but generally, the susceptible variety show a less control values than that of resistant one.

Gross growth parameters of both susceptible and resistant *Phaseolus vulgaris* plants, biologically controlled by *Streptomyces lydicus* against *Rhizoctonia solani* root rot disease

Results obtained from table (3) revealed that, there are a decrease in all plant growth parameters tested as root length, root fresh weight and total chlorophyll content in both susceptible and resistant *Phaseolus vulgaris* plant varieties in response to *Rhizoctonia solani* fungal infection as compared to their controls. However, the inhibitory effects of the pathogen on plant growth parameters was investigated by many authors (Hamad *et al*., 2001). In contrast, the use of a biocontrol agent, *Streptomyces lydicus* with resistant one. Interestingly, these results also run parallel to that obtained by both Rodriguez and Cotes in 1999 as they proved that plant treatment by biocontrol agent can significantly activate all of the plant physiological activities. Amazingly, the results obtained, collectively showed that the response percentage of the susceptible variety to all of the biological control treatments measured was higher than that of resistant variety.

Protein bands pattern in the electrophoregram of the eight treatment sample tested

Many biochemical studies have been carried out to investigate the metabolic changes associated with the occurance of plant defence reactions (Dolores *et al*., 1998 and Hamad *et al*., 2001). However, our qualitative analysis of the protein pattern was determined on the base of the number, density, molecular weight and reproducibility on SDS-PAGE. Bands with the same mobility were treated as identical fragments. But weak bands with negligible density and smear bands were both excluded from final analysis. However, the electropherogram of the eight treatment samples exhibited the presence of 25 protein bands with molecular weight ranged between 13-158 KDa. On the other hand, the protein bands of the eight treatment samples were varied in number and density of bands whereas S1, S2, S3, S4, S5, S6, S7 and S8 were revealed 18, 13, 16, 12, 14, 13, 13 and 16 protein bands respectively. The variability analysis of the eight samples showed some polypeptides bands absent or/and present in some habitat (polymorphic band; 93, 89, 67, 56, 54, 42, 37, 32, 30, 28, 27, 22, 19, 17 and 16) with percentage of 60%.

Table 2. A greenhouse biological control of *Rhizoctonia solani* root rot disease on *Phaseolus vulgaris* susceptible and resistant plants by *Streptomyces lydicus*

Treatments	Class					Disease index	Infection %
	0	1	2	3	4		
S1: Untreated susceptible plant/control (S.C)	15	2	1	0	0	6	17
S2: (S.C) + Biocontrol *St. lydicus*	16	2	0	0	0	3	11
S3: Infected plants with *Rhizoctonia solani* (I.S.P)	1	0	2	6	9	81	94
S4: (I .S.P) + Biocontrol *St. lydicus*	14	3	1	0	0	7	22
S5: Untreated resistant plant/control (R.C)	17	1	0	0	0	1	6
S6: (R.C) + Biocontrol *St. lydicus*	18	0	0	0	0	0	0
S7: Infected resistant plants with *Rhizoctonia solani* (I.R.P)	11	4	2	1	0	15	39
S8: (I.R.P) + Biocontrol *St. lydicus*	17	1	0	0	0	1	6

Table 3. Gross growth parameters of both susceptible and resistant *Phaseolus vulgaris* plants, biologically controlled by *Streptomyces lydicus* against *Rhizoctonia solani* fungal pathogen

Treatments	Root length / cm	Root fresh weight /gm	Total chlorophyll A+B (mg/g fresh weight)
S1: Untreated susceptible plant/control (S.C)	6.2	10.1	23.3
S2: (S.C) + Biocontrol *St. lydicus*	6.6	10.5	26.1
S3: Infected plants with *Rhizoctonia solani* (I.S.P)	3.7	6.8	11.4
S4: (I .S.P) + Biocontrol *St. lydicus*	7.0	11.6	20.0
S5: Untreated resistant plant/control (R.C)	5.8	9.7	25.1
S6: (R.C) + Biocontrol *St. lydicus*	7.2	11	27.0
S7: Infected resistant plants with *Rhizoctonia solani* (I.R.P)	5.5	9.2	18.5
S8: (I.R.P) + Biocontrol *St. lydicus*	5.6	10	23

Results obtained from both table (4) and Fig. (1 & 2) revealed that, the eight treatments samples characterized by the presence of 6 monomorphic common polypeptide bands with MW of 80, 60, 47, 23, 14 and 13 KDa with percentage of 24%. However, four unique bands were recorded with percentage of 16%, three of them were detected in S1 with MW of 158, 108 and 99 KDa. And the fourth band was detected in S8 with MW of 18 KDa. Interestingly, the obtained results are in agree with the view other authors as the new protein band found in S8 (the resistant infected variety treated with the biocontrol), may be related to the metabolic changes associated with the defence response, and from the metabolic point of view the infected plant cell can produce certain types of proteins called pathogen related protein (PR), that may play an important role in plant defence mechanism, and most of them show antifungal activity (Brigitte and Metraux, 1998).

The genetic distance between different treatment samples detected by qualitative analysis of the protein pattern of the eight samples tested

Genetic distance (GD), was measured as the distance difference between each sample. Since, the highest GD was detected between S8 and S1 samples which represent 0.37. On the other hand, the lowest distance was 0.20 between S5 and S4 as well as between S7 and S6 samples. These results exemplified in table (5) show that, there's a great variation between these samples in genetic content. However, according to the obtained results we can arrange the relations between the susceptible variety treatment sample according to the control in a descended distance as ,(S1 to S2 /0.36, S4 /0.31, and to S3 /0.28) as well as, the resistant variety treatment samples in a descended distance also display (S5 to S8 /0.28, S7 /0.26 and to S6 /0.22).

The Genetic similarity between different samples detected by qualitative analysis of the protein pattern of the eight treatment samples

Data obtained from figure (3) & table (6), clarified that the genetic similarity of the eight samples detected by qualitative analysis of the protein pattern similarity ranged between 58.1% and 84.7%. However, the obtained results are in consistent with Mauch-Mani and Metraux (1998) as they mentioned that, the activation of the disease resistance response in a host plant frequently requires the interaction of a plant gene product, with a corresponding pathogen derived signal and the

products of the resistance genes from diverse plant species, show remarkable structural similarity. However, from the qualitative analysis of protein pattern of the eight samples, we can arrange relations between the susceptible variety treatment samples according to the control in a descended protein pattern similarity as (S1 to S3 /76.5, S4 /66.7 and to S2 /58.1) as well as the resistant variety treatment descended protein pattern similarity also display (S5 to S6 /81.5, S7 /74.1 and to S8 /73.3).

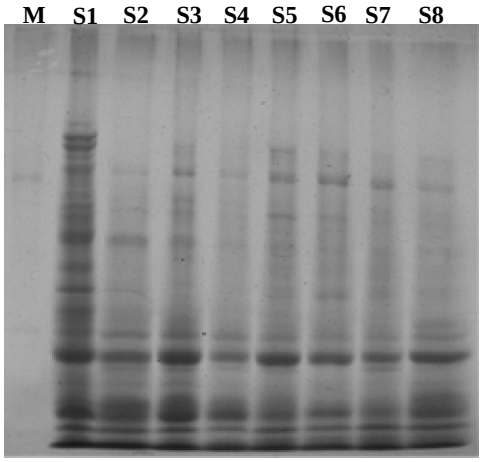

Figure 1. SDS-PAGE protein patterns of eight samples. Lane M: Protein marker, Lanes S1 to S8

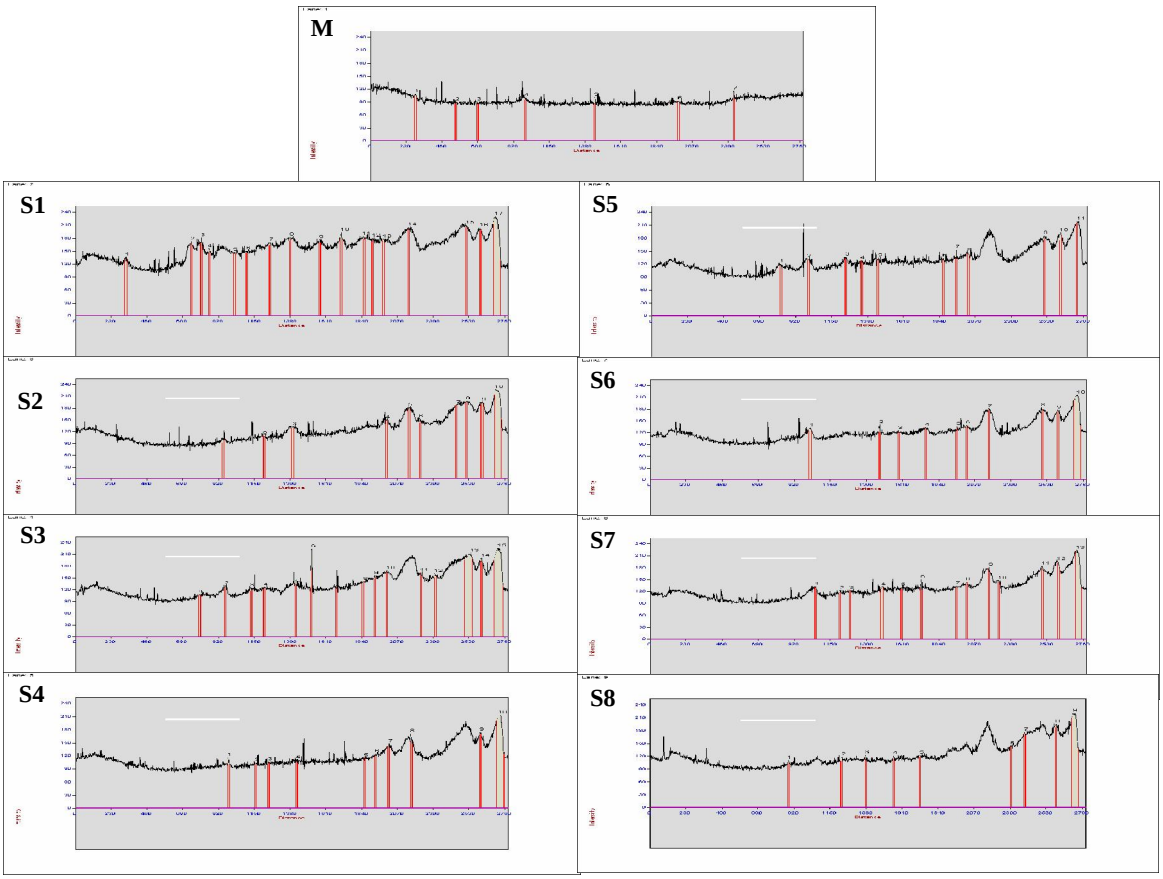

Figure 2. Protein bands pattern in the electrophoregram of (M: marker + eight treatment samples from S1 to S8)

Table 4. Scoring sheet of protein bands pattern in the electrophoregram of the eight treatment samples

Band No.	M.Wt (KDa)	(S1) Lan.2		(S2) Lan.3		(S3) Lan.4		(S4) Lan.5		(S5) Lan.6		(S6) Lan.7		(S7) Lan.8		(S8) Lan.9		polymorphism
		Band score	Relative protein content	Band score	Relative protein content	Band score	Relative protein content	Band score	Relative protein content	Band score	Relative protein content	Band score	Relative protein content	Band score	Relative protein content	Band score	Relative protein content	
1	158	1	2.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Unique
2	108	1	0.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Unique
3	99	1	7.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Unique
4	93	1	5.4	0	0.0	1	1.7	0	0.0	1	7.2	0	0.0	0	0.0	0	0.0	Polymorphic
5	89	1	1.6	0	0.0	0	0.0	0	0.0	0	0.0	1	0.9	0	0.0	1	2.7	Polymorphic
6	80	1	0.8	1	1.2	1	3.7	1	2.6	1	10.6	1	7.8	1	5.1	1	2.2	Monomorphic
7	67	1	1.0	0	0.0	1	0.8	1	1.5	0	0.0	0	0.0	0	0.0	0	0.0	Polymorphic
8	60	1	1.8	1	0.9	1	0.9	1	1.2	1	5.6	1	1.2	1	1.9	1	1.6	Monomorphic
9	56	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.2	1	1.5	1	0.9	Polymorphic
10	54	0	0.0	0	0.0	0	0.0	1	0.5	1	0.8	0	0.0	0	0.0	1	1.2	Polymorphic
11	47	1	4.7	1	7.5	1	1.3	1	1.3	1	1.8	1	2.1	1	4.3	1	1.6	Monomorphic
12	42	1	2.4	1	2.5	1	11.7	0	0.0	1	1.5	1	0.7	0	0.0	1	1.2	Polymorphic
13	37	1	5.4	0	0.0	1	0.1	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	Polymorphic
14	32	1	2.6	1	6.8	1	1.8	0	0.0	1	1.8	1	2.5	1	4.0	1	2.0	Polymorphic
15	30	1	0.9	0	0.0	0	0.0	1	0.5	1	1.8	0	0.0	0	0.0	0	0.0	Polymorphic
16	28	1	1.6	0	0.0	1	1.6	1	1.1	1	2.6	1	1.4	1	0.8	1	2.2	Polymorphic
17	27	0	0.0	1	4.6	1	2.2	1	4.9	1	4.6	1	1.7	1	2.2	1	2.8	Polymorphic
18	23	1	6.1	1	9.9	1	4.4	1	9.6	1	13	1	8.8	1	6.5	1	10.3	Monomorphic
19	22	0	0.0	1	1.5	1	0.6	0	0.0	0	0.0	0	0.0	1	0.4	0	0.0	Polymorphic
20	19	0	0.0	1	0.5	1	0.1	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7	Polymorphic
21	18	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	5.6	Unique
22	17	0	0.0	1	5.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	5.0	Polymorphic
23	16	1	3.8	1	4.0	1	19.4	1	8.4	1	10.9	1	8.0	1	10.1	0	0.0	Polymorphic
24	14	1	0.8	1	3.9	1	1.0	1	1.1	1	9.9	1	4.7	1	7.0	1	3.2	Monomorphic
25	13	1	51.0	1	51.4	1	48.7	1	67.3	1	27.9	1	59.0	1	55.2	1	56.8	Monomorphic
Total band score		18	100	13	100	16	100	12	100	14	100	13	100	13	100	16	100	

Table 5. The genetic distance between different treatment samples detected by qualitative analysis of the protein pattern of the eight samples tested

Samples	S1	S2	S3	S4	S5	S6	S7	S8
S1	0.000							
S2	0.3606	0.000						
S3	0.2828	0.2236	0.000					
S4	0.3162	0.3000	0.2828	0.000				
S5	0.2828	0.2646	0.2449	0.2000	0.000			
S6	0.3000	0.2449	0.2646	0.2646	0.2236	0.000		
S7	0.3317	0.2449	0.2236	0.2646	0.2646	0.2000	0.000	
S8	0.3742	0.2646	0.3162	0.3162	0.2828	0.2236	0.3000	0.000

Table 6. Genetic similarity between different treatment samples detected by qualitative analysis of the protein pattern of the eight samples tested

Samples	S1	S2	S3	S4	S5	S6	S7	S8
S1	100.0							
S2	58.1	100.0						
S3	76.5	82.8	100.0					
S4	66.7	64.0	71.5	100.0				
S5	75.0	74.1	80.0	84.7	100.0			
S6	71.0	76.9	75.9	72.0	81.5	100.0		
S7	64.6	76.9	82.8	72.0	74.1	84.7	100.0	
S8	58.8	75.9	68.8	64.3	73.3	82.8	69.0	100.0

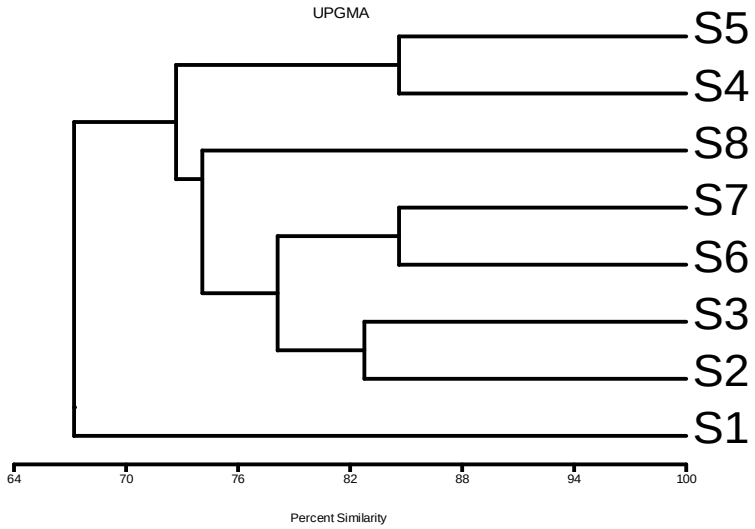

Figure 3. Dendogram obtained by cluster analysis based on presence/absence of matrix protein

Collectively, and from data obtained in figure (3) and tables (5 & 6) related to both genetic distance and similarity, results conclude that, the higher genetic distance samples is the lower genetic formula affinity and vice versa, so the highest genetic formula affinity was detected between both S4 & S5, and the lowest genetic formula affinity was detected between both S1 & S8, and this in return means that there are a great genetic similarity between the susceptible infected variety treated with biocontrol agent and the resistant control untreated variety S4 & S5, but not between the resistant infected variety treated with biocontrol agent and the susceptible control untreated variety S1 & S8,. More obviously, results conclude that the control of the protein synthesis is therefore a key problem in the resistance mechanism in plants and the proper control of susceptible variety will be equal to that of resistant one, and this may be the ultimate goal for saving a time, effort, and money to produce a new resistant variety annually. Finally, this study highlight on the need for extra work in this field for understanding more about the mechanism of pathogen related proteins (PR) in the resistance and in turn in the plant immune system that may eliminate the plant disease ghost from our life.

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1/21/2011