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ANTIGENIC DIFFERENCES BETWEEN CERTAIN UNINOCULATED AND INOCULATED RICE CULTIVARS WITH *PYRICULARIA ORYZAE* CAV.

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Abstract

Uninoculated and inoculated plants of three rice cultivars namely Reiho (blast-susceptible), GZ 2175-5-6 (Promising lines) and IR 28 (resistant) with *Pyricularia oryzae* were differentiated serologically by using double gel diffusion (DGD) and crossed immunoelectrophoresis (CIE) techniques. Differences in the antigenic structures were found between inoculated and uninoculated plants of Reiho and GZ 2176-5-6, since three and one antigens were not detected in the inoculated plants, respectively. Fourteen antigens were detected in the homologous reactions of uninoculated Reiho and GZ 2175-5-6, while 11 and 13 antigens were detected in the inoculated plants respectively. In contrast, no antigenic changes were found following inoculation of resistant cv. IR 28 since 14 antigens were detected in both homologous and heterologous reactions. On the other hand, the serological relationship between the three cultivars were determined according to the antigens common among them. IR 28 was serologically more related to GZ 2175-5-6 than Reiho, where 12 and 8 antigens were in common respectively. Results revealed that the antigenic differences between inoculated and uninoculated rice cultivars were associated with the cultivar susceptibility.

INTRODUCTION

Changes in protein constitution in plant (*Nicotiana tabacum*) responding with hypersensitivity to infection with tobacco mosaic virus (TMV) were reported by many investigators (Gianinazzi *et al.* 1970 and Van Loon and Van Kammen 1970).

Several proteins, absent in healthy plants were found to be formed at the onset of hypersensitivity. These proteins were named "b-proteins" by Gianinazzi *et al.* (1970) and "pathogenesis-related proteins" (PRs) by Antoniw *et al.* (1980). Very little is known about the origin of these proteins. The role of these proteins in acquired resistance has been questioned as they appear to be constituent host proteins of healthy flowering tobacco plants with senescent lower leaves (Fraser, 1981), with no apparent central role in acquired resistance (Fraser, 1982). On the other hand, Kassanis and White (1974) showed that the appearance of these new proteins and the formation of acquired resistance were inhibited by actinomycin D. Therefore, they suggested that these proteins are responsible for acquired resistance and might represent a kind of plant interferons. The serological studies carried out on RPs proteins detected in tobacco "Xanthi-nc" inoculated with TMV and stressed by chemicals showed that these proteins were identical and associated with the acquired resistance (El-Kady *et al.* 1989). The aim of the present work is to study the antigenic changes in the compatible and incompatible rice - *P.oryzae* combinations.

MATERIALS AND METHODS

A. Plants and pathogen:

Three rice cultivars, Reiho (susceptible), GZ 2175-5-6 (Promising line) and IR 28 (resistant) uninoculated and inoculated with *P. oryzae* were used in the present study. Three weeks old seedlings of each cultivar were inoculated with spore suspension of *P. oryzae* at the concentration of 10^5 spore/ml. The same methods were followed as indicated by Sehly *et al.* (1990).

B. Antigen preparation and immunization:

Plant antigens were prepared according to Antoniw and Pierpoini (1978) using a buffer containing 84 mM citric acid, 32 mM Na_2HPO_4, 14 mM mercaptoethanol and 6 mM L-ascorbic acid (pH 2.8). Fungal extract was obtained according to Ala El-Dein and El-Kady (1985). Protein content adjusted to 20 mg/ml. Rabbits were injected 10 injections (two per week) starting with 1 ml then increased by 0.5 ml for each following injection. Incomplete adjuvant was mixed with plant and fungal extract

(ratio 1:1). One week after the last injection, the rabbits were bled and immunoglobulins were isolated according to Harboe and Ingild (1973). Antibodies were absorbed for the reciprocal analysis procedure according to El-Kady *et al.* (1986).

C. Serological tests:

Double gel diffusion test was carried out according to Ouchterlony and Nilsson (1973). Crossed immunoelectrophoresis techniques were used according to Axelsen *et al.* (1973). Barbital buffer (pH 8.6, with ionic strength 0.03) was used. Ten um of the antigen preparations were electrophoresed in the first dimension at 10 V cm^{-1} for 1 h at 15°C and the second dimension was at 2 V cm^{-1} for 20 h.

RESULTS

Serological differences among rice cultivars inoculated or uninoculated with *P. oryzae*:

Three rice cultivars, Reiho as susceptible cv., IR 28 as resistant cv. and GZ 2175-5-6 as promising line, inoculated with *P. oryzae* or uninoculated, were compared serologically by using double gel diffusion (DGD) and crossed immunoelectrophoresis (CIE) techniques. The DGD test showed that one antigen was not detected in inoculated Reiho plants, but was detected in the uninoculated plants, in the homologous reactions (Table 1 and Fig. 1a,b). In the cross heterologous reactions between antibodies against inoculated and uninoculated Reiho and antigens of uninoculated and inoculated plants, the same antigens were detected.

No differences in the antigenic structures of uninoculated and inoculated plants of IR 28 cv. were observed when antigens of its uninoculated and inoculated plants were reacted with antibodies against inoculated and uninoculated plants of all the rice cultivars (Table 1).

Three antigens were found in common to IR 28 cv. and Reiho cv., while six antigens were common to IR 28 and GZ 2175-5-6. On the other hand, four antigens were found common to inoculated Reiho and inoculated GZ 2175-5-6, while five antigens were common to uninoculated plants of Reiho and GZ 1275-5-6 (Table 1).

One antigen was not detected in the inoculated plants of GZ 2175-5-6, when compared to the uninoculated ones, as six and seven antigens were detected respectively, in the homologous reactions (Table 1).

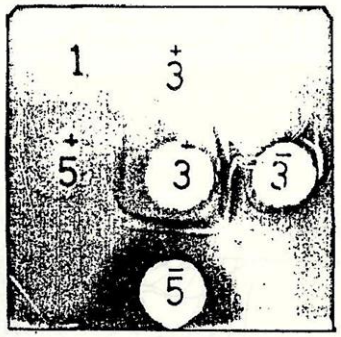
The present results clearly showed that antigenic differences occurred between uninoculated and inoculated plants of the rice cultivars, Reiho and GZ 2175-5-6, while no differences were found between uninoculated and inoculated plants of IR 28. Also, the results revealed that GZ 2175-5-6 was serologically related to both Reiho and IR 28 cvs, since four and six antigens were, respectively, found in common, while three antigens were found in common between Reiho and IR 28 cvs.

In the CIE technique, the numbers of precipitin bands detected in all the reactions were almost twice those detected in the DGD test. CIE showed that three antigens were undetectable in inoculated Reiho cv. in comparison with uninoculated plants (Table 1 and Fig. 2a,b). Eleven antigens were detected in the heterologous reactions (Fig. 3a). Furthermore, CIE with an intermediate gel showed these three

Table 1. Serological reactions among three uninoculated and inoculated rice cultivars by *P. oryzae* using the double gel diffusion test (DGD) and crosses immunoelectrophoresis (CIE).

Antigens / Antibodies		Number of precipitin peaks detected							
		Reiho		IR 28		GZ 2175-5-6			
		DGD		CIE		DGD		CIE	
		-	+	-	+	-	+	-	+
Reiho	-	5+S	5	11+3S	11	3	3	7	7	4+S	4	5+S	5
	+	5	5	11	11	3	3	7	7	4	4	5	5
IR28	-	3	3	7	7	6	6	14	14	3	3	14	14
	+	3	3	7	7	6	6	3	3	3	3	14	14
GZ 2175-5-6	-	4+S	4	8+S	8	6	6	14	14	4+S	4	12+S	12
	+	4	4	8	8	6	6	13	14	4	4	12	12

- = Uninoculated
+ = Inoculated
S = Antigens associated with susceptibility.



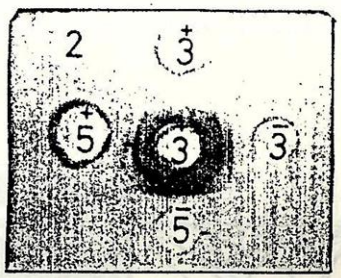


Fig. 1. Double gel diffusion (DGD) test,
Central wells contained antibodies against:
(a) Uninoculated Reiho cv.

(b) inoculated Reiho cv.

Peripheral wells contained antigens of:
3-: uninoculated Reiho
5-: uninoculated GZ 2175-5-6

3+: inoculated Reiho
5+: inoculated GZ 2175-5-6

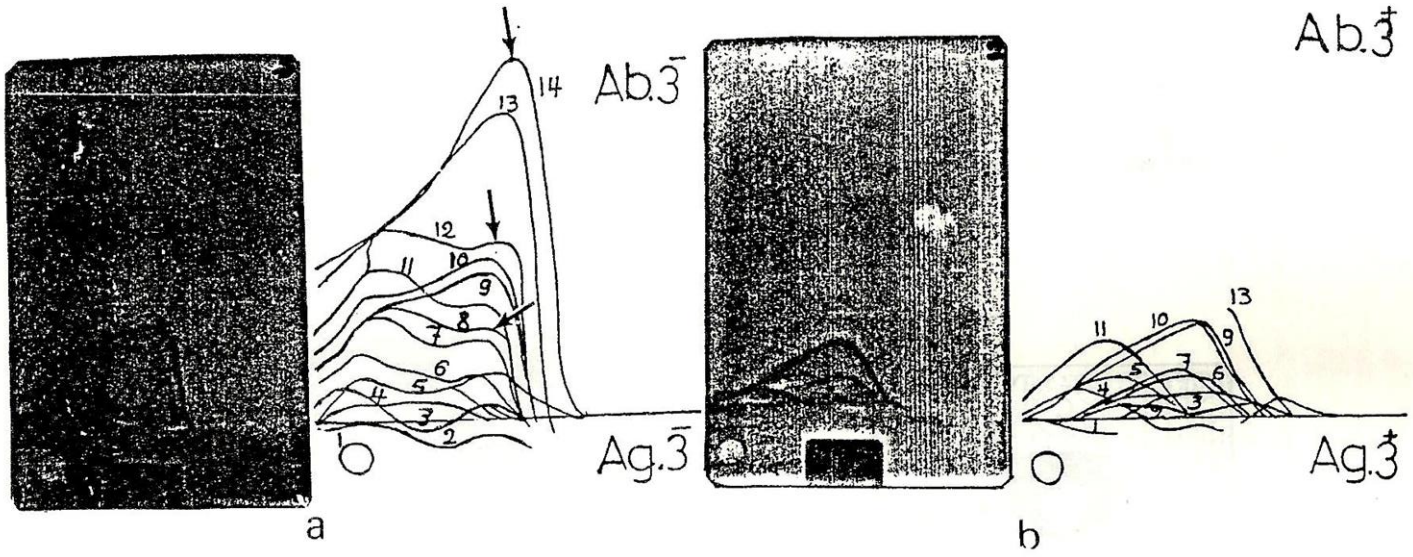


Fig. 2. Crossed immunoelectrophoresis (CIE) of uninoculated (a) and inoculated (b) Reiho by *P. oryzae*. Antigens of uninoculated (Ag. 3-) and inoculated (Ag. 3+) plants were electrophoresis. Upper gel contained antibodies against uninoculated (Ab. 3-) and inoculated (Ab. 3+) plants. Arrows indicate the antigenic differences between inoculated and uninoculated Reiho cv. Anodes at the right and the top.

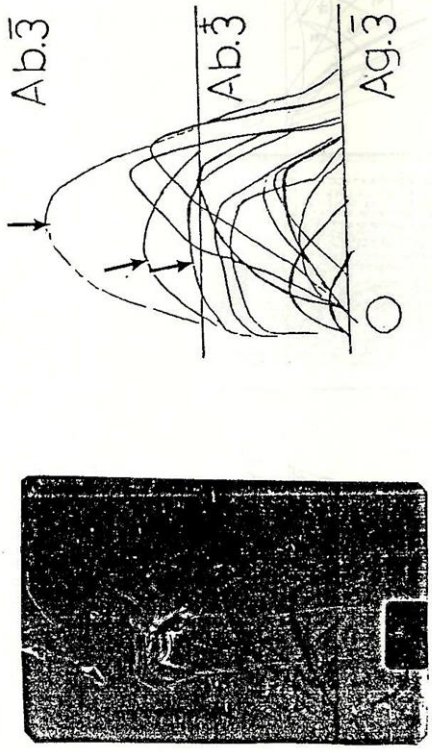


Fig. 3. CIE with an intermediate gel of Reiho cv. antigen-antibody reaction system. Antigens uninoculated (Ag. 3-) plants were electrophorized. Upper gel contained antibodies against uninoculated plants (Ag. 3-). The intermediate gel contained antibodies against inoculated plants (Ag. 3+). Arrows indicate the antigenic differences between inoculated and uninoculated plants.

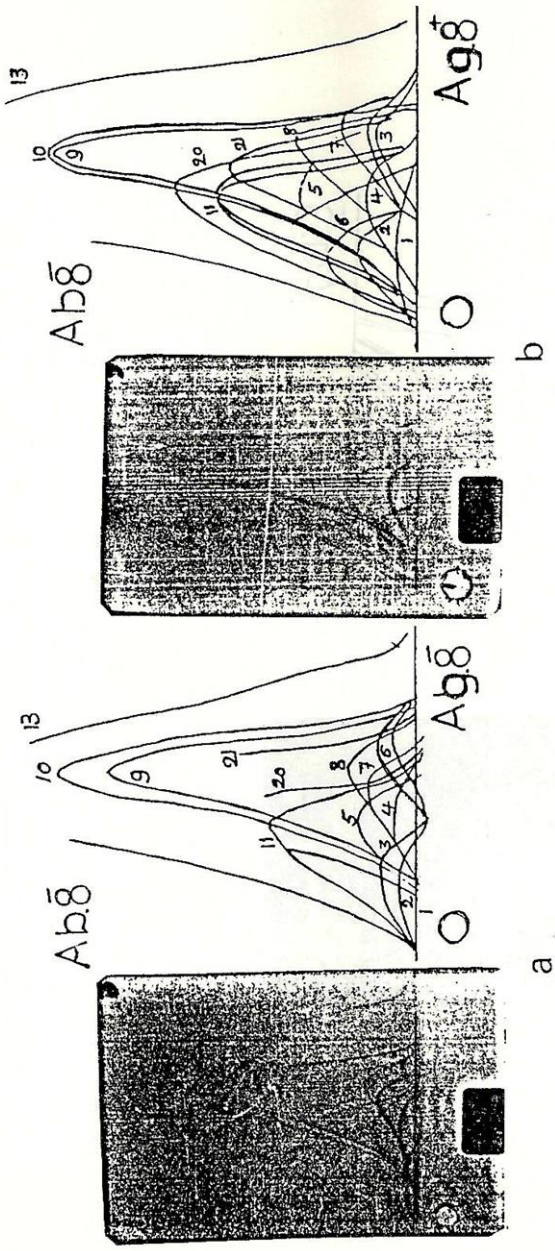


Fig. 4. CIE in homologous reaction of uninoculated IR28 (a) in heterologous reaction of uninoculated and inoculated IR28 (b). Antigens of uninoculated (Ag. 8-) and inoculated (Ag. 8+) were electrophorized. Upper gel contained antibodies against uninoculated plants (Ab. 8-). No antigenic differences were observed between uninoculated and inoculated IR28 cv.

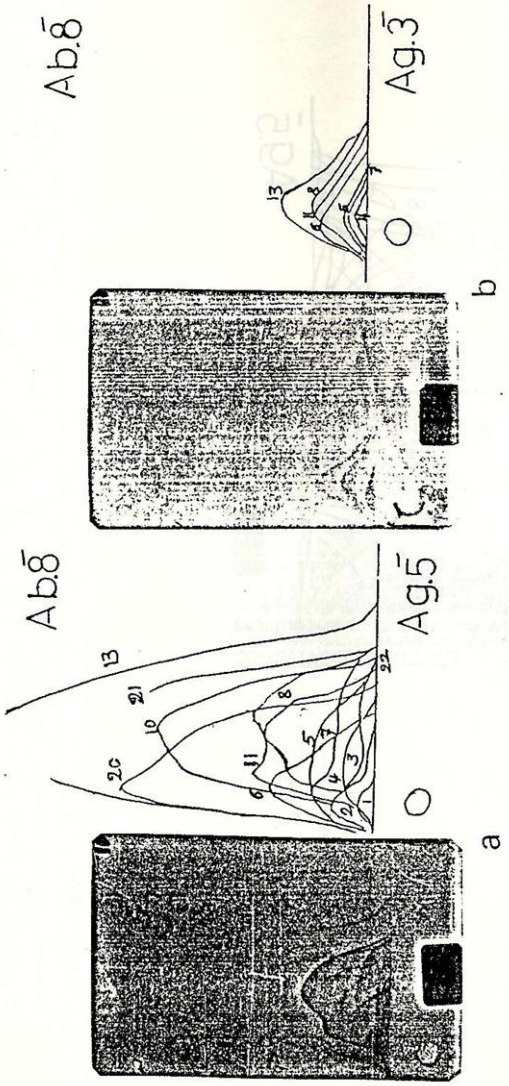


Fig. 5. CIE of antibodies against uninoculated IR28 with uninoculated GZ 2175-5-6 antigens (a) and with uninoculated Reiho antigens (b). Antigens of uninoculated Reiho (Ag. 3-) and uninoculated GZ 2175-5-6 (Ag. 5-) were electrophorized. Upper gel contained antibodies against uninoculated IR28 (Ab. 8-).

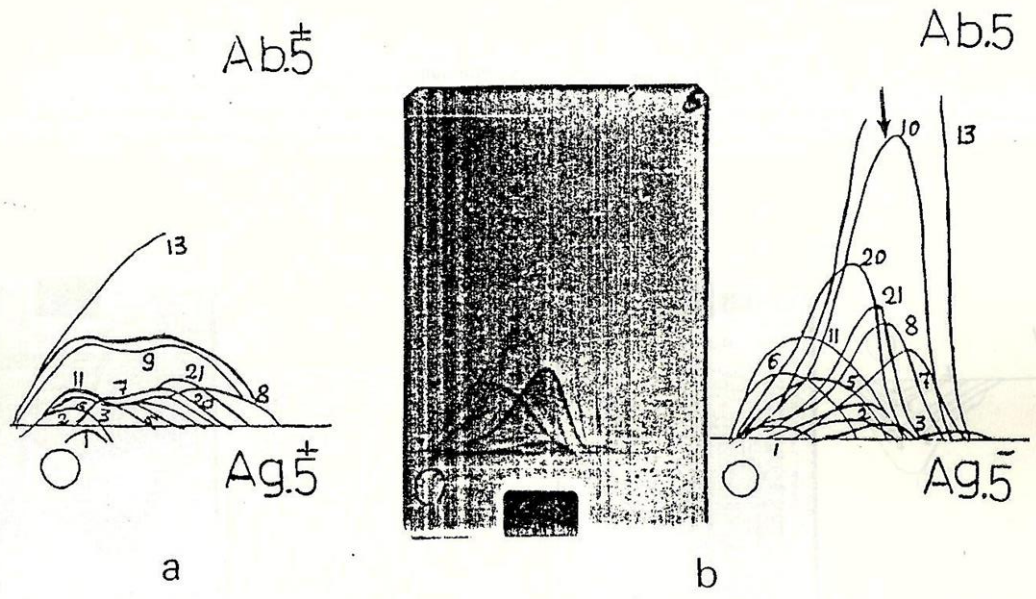


Fig. 6. CIE of inoculated (a) and uninoculated (b) GZ 2175-5-6 in homologous antigen-antibody reaction system. Antigens of uninoculated (Ag. 5-) and inoculated (Ag. 5+) plants were electrophorized. Upper gel contained antibodies against uninoculated (Ab. 5-) and inoculated (Ab. 5+) plants. Arrow indicates the antigenic differences between inoculated and uninoculated plants.

antigens with the uninoculated Reiho compared with the inoculated plants (Fig. 3b). Antibodies against uninoculated Reiho differentiated between uninoculated and inoculated GZ 2175-5-6, since one antigen was not detected in the inoculated plants, while thirteen antigens were found common in both inoculated and uninoculated plants (Table 1).

On the other hand, no antigenic differences were found between uninoculated and inoculated plants of all the tested cultivars when their antigens were reacted with antibodies against inoculated and uninoculated IR 28. Fourteen antigens were detected in the homologous reactions of uninoculated and inoculated IR 28 (Fig. 4a,b). On the other hand, fourteen and seven antigens were detected between antibodies against IR 28 (inoculated or uninoculated) and antigens of GZ 2175-5-6 and Reiho, respectively (Fig. 5a,b).

One antigen was not detected in inoculated GZ 2175-5-6 in comparison with uninoculated plants, where thirteen and fourteen antigens were found, respectively (Table 1) and Fig. (6a). Thirteen antigens, as indicated by reaction peaks were detected in the homologous antigen-antibody reaction of inoculated GZ 2175-5-6.

Generally, CIE technique showed that the serological relationship between GZ 2175-5-6 and IR 28 was more than those between Reiho and any of these cultivars. Also, the results revealed that three and one antigens were not detected in inoculated cultivars of Reiho and GZ 2175-5-6, respectively, when compared with uninoculated plants. No changes in the antigenic structures were found between inoculated and uninoculated IR 28. Reciprocal analysis procedure (Table 2) showed similar trends.

Table 1. Serological differences between uninoculated *P. oryzae* inoculated rice cultivars in the reciprocal analysis procedure using CIE.

Antibodies	Antigens used for absorption	Number of precipitin peaks detected					
		Reiho		IR 28		GZ 2175-5-6	
		-	+	-	+	-	+
Reiho -	Reiho -	0	0	0	0	0	0
	Reiho +	3S	0	0	0	S	0
	IR28 -	4+3S	4	0	0	2+S	2
	IR28 +	4+3S	4	0	0	2+S	2
	GZ 2175-5-6 -	2+2S	2	0	0	0	0
	GZ 2175-5-6 +	2+3S	1	0	0	S	0
	P. oryzae	9	9	0	0	8	8
Reiho +	Reiho -	0	0	0	0	0	0
	Reiho +	0	0	0	0	0	0
	IR28 -	4	4	0	0	2	2
	IR28 +	4	4	0	0	2	2
	GZ 2175-5-6 -	2	2	0	0	0	0
	GZ 2175-5-6 +	2	2	0	0	0	0
	P. oryzae	9	9	0	0	8	8
IR28 -	Reiho -	0	0	7	7	7	7
	Reiho +	0	0	7	7	7	7
	IR28 -	0	0	0	0	0	0
	IR28 +	0	0	0	0	0	0
	GZ 2175 -	0	0	0	0	0	0
	GZ 2175 +	0	0	0	0	0	0
	P. oryzae	9	9	0	0	8	8
IR28 +	Reiho -	0	0	7	7	7	7
	Reiho +	0	0	7	7	7	7
	IR28 -	0	0	0	0	0	0
	IR28 +	0	0	0	0	0	0
	GZ 2175-5-6 -	0	0	0	0	0	0
	GZ 2175-5-6 +	0	0	0	0	0	0
	P. oryzae	9	9	0	0	8	8
GZ 2175-5-6 -	Reiho -	0	0	4	4	4	4
	Reiho +	2	0	4	4	4+S	4
	IR28 -	S	0	0	0	0	S
	IR28 +	S	0	0	0	S	0
	GZ 2175 -	0	0	0	0	0	0
	GZ 2175 +	S	0	0	0	S	0
	P. oryzae	6	6	6	6	6	6
GZ 2175-5-6 +	Reiho -	0	0	4	4	4	4
	Reiho +	0	0	4	4	4	4
	IR28 -	0	0	0	0	0	0
	IR28 +	0	0	0	0	0	0
	GZ 2175-5-6 -	0	0	0	0	0	0
	GZ 2175-5-6 +	0	0	0	0	0	0
	P. oryzae	6	6	6	6	6	6

- = Uninoculated + = Inoculated S = Antigens associated with susceptibility.

DISCUSSION

This work could be the first attempt in this respect in Egypt from the available literature.

Results of the present work indicated that, three antigens were lost in the susceptible Reiho cv., following infection by a compatible race of *P. oryzae*, while only one antigen was lost in GZ 2175-5-6 by inoculation. No differences were found between healthy and inoculated plants of the resistant IR 28 cv.

However, GZ 2175-5-6 was serologically related to both Reiho and IR 28 cvs as Japonica and Indica types. Therefore, GZ 2175-5-6 may have acquired its resistant reaction to most tested races from its parents (Calrose 76 and Nagina cvs). Since 13 and 8 antigens were detected in common between GZ 2175-5-6 and IR 28 and GZ 2175-5-6 and Reiho, while only 7 antigens were common in both Reiho and IR 28.

It could be concluded from serological test that such a new technique in this field of study is beneficial in differentiating between resistant and susceptible cultivars (El-Kady *et al.* 1989). This finding could save time in selecting varieties or promising lines. However, more work is needed on this point of study in future to be applied widely in breeding programs.

In the present study, some antigens (or proteins) were lost in inoculated plants (compatible interaction) and were found in common with *P. oryzae* (data under publication) indicate that these antigens may be associated with cultivar susceptibility. This could also be explained by that the pathogen may cause some damage to certain genes associated with susceptibility. The hosts antigens that disappear upon inoculation and successful establishment of the pathogen are assumed to play some role in a reaction leading to susceptibility. This could be similar to the recognition process and could also results from the transformation of such proteins to some nonantigenic components. In contrast, in case of hypersensitive reactions, some new proteins (antigens) were detected in inoculated or treated plants in comparison with healthy ones and proved their role in the acquired resistance (Hornok and Kiraly 1984 and El-Kady *et al.* 1989).

REFERENCES

1. Ala El-Dein, O. and S. El-Kady. 1985. Immunological Comparison Between Isolates of *Botrytis cinerea* Pers. by Using Crossed Immunoelectrophoresis Techniques. Acta. Phytopath. Acad. Sci. Hung., 20: 283-290.
2. Antoniw, J.F. and W.S. Pierpoint. 1978. The purification and properties of one of the "b" proteins from virus-infected tobacco plants. J. Gen. Virol., 39: 343-350.
3. Antoniw, J.F., E.C. Pitter, S.W. Pierpoint and L.C. Van Loon. 1980. Separation and comparison of three pathogenesis related proteins from two cultivars of virus infected tobacco plants. J. Gen. Virol., 47: 79-87.
4. Axelsen, M.H., J. Kroll and B. Weeke. 1973. A manual of quantitative immunoelectrophoresis methods and application. Universitats of Laget, Oslo.
5. El-Kady, S., Morai Hevesi, G. Somlyai and Z. Klement. 1986. Immunoelectrophoretic studies of three Pseudomonas syringae Pathovars. Acta Phytopath. Entomol. Hung., 21: 93-98.
6. El-Kady, S., R. Gaborjanyi and Z. Kiraly. 1989. Serological comparison of antigens related to local virus and bacterial infections and a specific stresses in tobacco leaves. J. Phytopath., 126: 69-78.
7. Fraser, R.S. 1981. Evidence for the occurrence of the "pathogenesis-related" proteins in leaves of healthy tobacco plants during flowering. Physiol. Pl. Path., 19: 69-76.
8. Fraser, R.S. 1982. Are "Pathogenesis-related" proteins involved in acquired systemic resistance to tobacco plants to tobacco mosaic virus? J. Gen. Virol., 58: 305-313.
9. Gianinazzi, S., C. Martin and J.C. Vallee. 1970. Hypersensibilite aux virus, temperature et proteines solubles chez le Nicotiana Xanthi-n.c. Apparition de nouvelles macromolecules lors de la repression de la synthese virale. Compt. Rend. Acad. Sci. D 270, 2383 a 2386.
10. Harboe, N. and A. Ingild. 1973. Immunization, isolation and immunoglobulins, estimation of antibody titre. Scand. J. Immunol. 2, Suppl., 1: 161-164.
11. Hornok, L. and Z. Kiraly. 1984. Changes in the antigen composition of the upper leaves of cucumber plants after inducing systemic acquired resistance by inocu-

lation with *Colletotrichum lagenarium*. Physiol. Plant. Pathol., 25: 171-179.

12. Kassanis, B. and R.F. White. 1974. Inhibition of acquired resistance to tobacco virus by actinomycin D. J. Gen. Virol., 25: 323-324.
13. Loon, L.C. Van and A. Van Kammen. 1970. Polyacrylamide disc electrophoresis of the soluble leaf proteins from *Nicotiana tabacum* var. "Samsun" and "Samsun NN". II-Changes in protein after infection with tobacco mosaic virus. Virology, 40: 199-211.
14. Ouchterlony, O. and A.L. Nilsson. 1973. Immunodiffusion and immunoelectrophoresis. In: D.M. Weir (ed), Handbook of Experimental Immunology. Blackwell Scientific Publications, Oxford.
15. Sehly, M.R., Z.H. Osman, H.A. Mohamed and A.O. Bastawisi. 1990. Reaction of some rice entries to *Pyricularia oryzae* cav. and race picture in 1988. The Six Congress of Phytopathology, Cairo, March 1990: 161-172.

الإختلافات الأنتيجينية بين أصناف الأرز المعدية وغير المعدية بالفطر بيريكيولاريا أوريزا

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تم التفريق سيرولوجيا بين ثلاثة أصناف من الأرز هى ريهو (قابل للإصابة) والسلالة المبشرة GZ 2175-5-6 (تحت الإختبار) والصنف IR 28 (مقاوم) لنباتات معداه وأخرى غير معداه بالفطر المسبب لمرض اللفحة *Pyricularia oryzae* وقد استخدم فى ذلك طريقتين هما طريقة الأنتشار المزدوج فى الاجار doubel gel diffusion (DGD) وطريقة Crossed immunoelectrophoresis (CIE) وقد وجدت فروق فى التركيبات الأنتيجينية بين النباتات المعداه والغير معداه لكل من الصنف ريهو والسلالة GZ 2175-5-6 لإختفاء ثلاثة أنتيجينات وأنتيجين واحد فى النباتات المعداه لكلا الصنفين على الترتيب. حيث أن أربعة عشرة أنتيجين وجدت فى التفاعلات المتجانسة للنباتات الغير معداه لكلا الصنفين ، بينما ظهر أحدى عشر ، ثلاثة عشر أنتيجين فى النبتات المعداه لهما. ومن ناحية أخرى لم يلاحظ أى إختلافات أنتيجينية بين النباتات المعداه والغير معداه للصنف المقاوم IR 28 ، حيث أن أربعة عشر أنتيجين وجدت فى جميع التفاعلات الخاصة بهذا الصنف. كما تحددت درجة القرابة السيرولوجية بين الأصناف الثلاثة تبعا للانتيجينات المشتركة بينها. فكان الصنف IR 28 أكثر قربا من الناحية السيرولوجية للسلاسة GZ 2175-5-6 عنه للصنف ريهو ، حيث وجد اثنى عشر، وثمانية انتيجينات مشتركة بينها على التوالى. وبصفة عامة أظهرت النتائج درجة علاقة الاختلافات الانتيجينية بين النباتات المعداه والغير معداه من أصناف الأرز ودرجة حساسية هذه الأصناف للاصابة بالفطر *P. oryzae*.