Investigations on serological relationship of virus Y strains

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The virus Y was described for the first time in 1920 by Orton and since then has been investigated by a number of scientists because of its special economical importance. Crops from potato plants, infected with this virus are known to be very low, and sometimes they are lacking at all [7, 16, 17]. Bawden and Kanssanis [2, 3] isolated various strains of this virus and prepared specific, diagnostic antisera. They pointed out for the serological relationship between different strains. Bartels [1] on the other hand stated, that strains belonging to different groups differ also greatly in serological properties.

In spite of the present knowledge concerning serological properties of different viruses, their serodiagnostic accuracy in agglutination and precipitin tests cannot be regarded as fully satisfactory. The present work aimed to examine the serological relationship of three strains of virus Y isolated in Roumania, in order to precise their serodiagnostics. For this purpose use was made of the complement fixation test for which optimal proportions of all components were established. A thorough analysis of physicochemical and some biological properties of the examined virus strains made them included into the groups of normal (Y^O) and necrotic (Y^N) strains [6, 9-11), two different strains being defined in this last group.

MATERIAL AND METHODS

The strain Y^O was isolated from the potato variety Osława, the strain Y^N from the variety Ackersegen, and the strain Y_1^N from the variety Bintje. Plants of the tobacco variety Samsun were used for the preparation of antigens of the examined virus strains, applied for the immunisation of rabbits and for carrying out serological reactions *in vitro*. Purification of the virus was performed by means of cold salt precipitation with ammonium sulphate in the moment when virus reached its maximal concentration in infected tobacco leaves.

Equal leaf weights were taken for purification of all strains. Rabbits were immunised according to the following procedure: antigens were introduced intramuscularly with the Freund's adjuvant four times, 8-10 days elapsing between individual injec-

tions. Totally about 5 mg of virus protein was introduced into each rabbit. Antigens and antisera were standardized by means of the cross titration method [8] performed in test tubes and on plastic slides with the use of the complement fixation reaction. The complement fixed in 4 % ortoboric acid and 10% NaCl, diluted to 1/10 was titrated in increasing amounts from 0.05, 0.1, 0.15 up to 0.5 ml at a total volume of 2.0 ml, in the presence of the examined serum (0.2 ml) and hemolytic system (1 ml). The first dilution of the complement at which hemolysis equaled 100% was taken for the complement titre. Subsequently 1.5 complement units were used in reactions. The hemolytic serum was titrated in the presence of complement excess according to scheme 1.

Scheme 1. Titration of the hemolytic system

Components	Dilution of the hemolytic serum					Complement control	Erythrocytes control
	1/500	1/1000	1/2000	1/2500	1/3000		
	volume of components ml						
Hemolytic serum	1	1	1	1	1	—	—
Erythrocytes 1.5%	1	1	1	1	1	1	1
Complement 1/10	0.5	0.5	0.5	0.5	0.5	0.5	—
Saline	—	—	—	—	—	1	15

Results of the test were estimated after the tubes were kept in water bath of 37°C for 30 minutes. The highest dilution of the serum which caused a complete hemolysis of the blood corpuscles of the ram was taken as the serum titre. For complement fixation 4 units of homologous antiserum were used.

The ram erythrocytes used in the test were freshly prepared and thouroughly washed in saline by threefold centrifugation at low speed. 1.5% suspension of those erythrocytes was applied in the hemolytical system.

The volume of the components in the hemolytical system equaled 0.4 ml when the complement fixation test was performed in test tubes, and 0.2 ml when the reaction was performed on plastic slides. The hemolytic system was sensitised for 30 minutes at 37°C before being used for the complement fixation.

The examined serum was inactivated at 56°C within 30 minutes. For complement fixation 4 units according to the titre of each of the examined virus strains were used. The test in the tubes was carried out at a total volume of 1 ml according to scheme 2.

Parallel to the main reaction following control reactions were carried out: control of antigens from normal plant proteins, control of normal rabbit serum, control of the complement, and finally control of the hemolytic system.

The hemolytic ability of the antigen was tested by using its double dose for the reaction with the hemolytic system without complement whereas anti-complement properties were examined by using its double dose in the presence of the complement and the hemolytic system.

Scheme 2. Complement fixation in test tubes

Components	Dilution of antiserum					Antigen control	Antiserum control
	1/2	1/4	1/8	1/16	1/1024		
	volume of components ml						
Inactivated antiserum at 56°C, 30 min	0.2	0.2	0.2	0.2	0.2	—	0.25
Antigen 4 units acc. to titre	0.2	0.2	0.2	0.2	0.2	0.4	—
Complement 1.5 units acc. to titre	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Saline	—	—	—	—	—	—	0.15
	Water bath 37°C for 45 minutes						
Hemolytic system	0.4	0.4	0.4	0.4	0.4	0.4	0.4

Scheme 3. Control reactions

Components	Antigen from normal plant proteins	Normal rabbit serum	Complement control	Hemolytic serum
	volume of components ml			
Antigen 4 units	0.4	—	—	—
Antiserum	—	0.25	—	—
Saline	—	0.15	0.4	0.6
Complement 1.5 units	0.2	0.2	0.2	—
Hemolytic serum	0.4	0.4	0.4	0.4
Total volume	1.0	1·0	1.0	1.0

Table 1

Titres of virus Y antisera before and after cross absorbtion

Antisera	Titres of antisera before cross absorbtion			Absorbent strain	Titres of antisera after cross absorbtion		
	virus test strain				virus test strain		
	Y^0	Y^N	Y_1^N		Y^0	Y^N	Y_1^N
Y^0	1024	256	256	Y^N	128	0	0
				Y_1^N	256	2	0
Y^N	128	512	256	Y^0	0	128	128
				Y_1^N	2	8	0
Y_1^N	64	128	256	Y^0	0	64	64
				Y^N	4	0	0

Reactions of cross absorbtion were carried out according to the modified method of Matthews [12]. A tiny volume of concentrated heterologous virus was added to undiluted antisera of virusses Y^O, Y^N, and Y_1^N in order to avoid dilition of the antiserum. This mixture was kept in a water bath at 37°C for two hours and subsequently centrifuged at 6000 r. p. m. for 30 min. The resulting supernatant was used for titration of each antiserum with homologous viruses by means of complement fixation. Final titres were compared with those obtained before absorbtion in the same reactions (Table 1).

DISCUSSION OF RESULTS

The present investigation was an attempt to examine serological properties of potato virus Y strains in order to obtain supplementary data concerning their antigen structure. The existance of serological relationship between the normal and necrotic virus Y strains was already reported by Bartels [1], Klinkowski and Schmelzer [14], and many others, using the precipitin test. In our experiment that kind of reaction was not applied. It was replaced by the complement fixation in order to avoid the negative influence of aggregation of bacilliform particles of the examined virus, what makes the evaluation of the results concerning the size of the macroprecipitate, difficult.

Applying the complement fixation test the established fact of the occurrence of microprecipitate formed in the first phase of the reaction by means of the hemolytic system permitted to evaluate the obtained results with a high degree of reliability. The technique of complement fixation test adopted by us for the examined system of antigen-antibody (Scheme 2), as well as the method of complement titration in the presence of a qiven antiserum made it possible to avoid anticomplement reactions obtained by some authors who worked with plant viruses using other schemes of complement fixation [13]. In our experiment best results were obtained when the complement was fixed in test tubes.

It should be emphasised, that obtaining high titres was not attempted by the preparation of strain specific sera in order to avoid unspecific reactions which usually take place in case of a longlasting immunisation of animals [5, 12, 15]. Titres of sera for each strain obtained in homologous and heterologous reactions are compared in Table 1. As is seen from these data, all the examined strains belong to the same serotype and contain both common and individual antigenic fractions. This is indicated by the difference in titres in homologous and heterologous reactions [4].

Titres of antiserum Y^O with homologous and heterologous strains Y^N and Y_1^N were 1024, 256 and 256 respectively. Titre of the antiserum Y^N with the homologous virus strain was 512, whereas with heterologous strains Y_1^N and Y^O was respectively: 256 and 128. Titres of the Y_1^N antiserum with the strains: Y_1^N, Y^N and Y^O were 256, 128 and 64.

In all three kinds of specific antisera obtained for the three virus Y strains, the presence of three types of antibodies reacting with both homologous and heterologous strains have been stated. The presence in the serum of antibodies of different

specificity reflects most probably respective differences in the antigenic structure of the examined strains. The titres of sera specific for necrotic strains (both homologous and heterologous) are almost identical, what indicates to a closer serological relationship between them, than with strains of the normal group.

The reaction of cross absorbtion permitted to draw some conclusions concerning the degree of relationship between the examined strains. The quantitative ratio of antibodies common for a group of strains and specific for each of the examined virus Y strains was determined according to the data characterising the decrease of the antiserum titre in reaction of cross absorbtion. A decrease of the titre of antisera from 512 to 8, and sometimes even to 0 (full absorbtion) in cross reactions between both necrotic strains shows that only a small part of antibodies (ca. 1/64) in the Y^N antiserum are able to react only with that strain.

The titres of antiserum specific for the normal strain Y^O revealed before absorbtion (1024) and after absorbtion by antigenes of necrotic strains (128 and 256) indicate to the fact, that 1/8 and 1/4 of the respective antigens in each serum is individually specific for a homologous strain.

As can be seen from the obtained data it seems aimful to apply strain specific sera for diagnosis of both normal and necrotic strains of potato virus Y in order to avoid falsely negative results in cases when concentration of heterologous strains in the investigated material is low. The serological marker indicating to the presence of many antigenic determinants of the necrotic virus Y strains gives evidence of a closer genetical relationship between them, than between necrotic and normal strains.

SUMMARY

Data concerning determination of the dgree of serological relationship of three strains of virus Y isolated in Roumania in order to precise trheir serodiagnostics are presented in the present paper.

The normal virus Y strain (Y^O) was isolated from the potato variety Osława, whereas the necrotic strains: Y^N and Y_1^N from the varieties Ackersegen and Bintje.

In order to determine the degree of relationship between the investigated strains, specific antisera were prepared for each of them. All serological tests were performed by the method of complement fixation, according to the scheme adapted individually for each antigen-antibody system. This eliminated the influence of antigen aggregation on the evaluation of results. Antigens were obtained with the salt precipitation method. Cross titration was applied for the determination of antigen titres.

On the basis of cross absorbtion tests a closer serological relationship was stated between two examined necrotic strains. Titres of homologous antisera decreased from 512 to full absorbtion respectively. A more remote relationship was stated between the normal and necrotic virus Y strains. In this case titres of homologous antisera decreased only 4 to 8 times.

In spite of the presence of common antigen groups in the examined strains, strain specific sera should be used for serodiagnostics of strains Y^O and Y^N in order to avoid falsely negative results when the heterologous virus Y strain occurs at low concentration.

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