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Some experiences in the identification of *"poty"* potato viruses by ELISA

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Abstract: The paper presents studies on the serological relatedness of potato viruses Y and A on the basis of extinction E_{405} values in ELISA.

It has been assessed that in 100% cases, in which only the necrotic strain of potato virus Y (PVY^N) was present, the use of IgG and EIgG for potato virus PVA resulted in the increased extinction (E_{405}) value. This value was twice the amount of the value in negative control 60 minutes after reading the reaction, increasing with the function of time. This phenomenon was not recorded when using the same antibodies in the samples in which PVY was absent.

Key words: potato virus Y; potato virus A; ELISA technique; serological relatedness.

Introduction

The enzyme-linked immunosorbent assay (ELISA) is a reliable technique for the identification of plant viruses, and thereby the potato viruses. Since its description by Clark and Adams (1977) , it has become irreplaceable in the routine identification of potato viruses.

It has been assessed that some viruses have identical amino acid residues in a part of the polypeptide chain of protein subunits (Shukla and Ward, 188), and therefore ELISA can detect the common epitopes of virus antigens (Tošić et al.,1990). Thus in the identificaton of a virus in ELISA, which is serologically related to the virus present in the sample, the increase in extinction (E_{405}) occurs without this virus being even present. Therefore, it is sometimes doubtful if a

positive or negative reaction is in question. Thus, tests by ELISA enable the assessment of serological relatedness between different viruses on the basis of reaction intensity.

Testing various plant material by ELISA, we have found out that in all the samples in which potato virus Y (PVY) when using IgG and EIgG for potato virus A (PVA), in most cases the extinction (E_{405}) value increased. The analysis of tests from several experiments revealed the increase in the extinction value (E_{405}) mainly in the cases where PVY^N was present. These values did not exceed (60 minutes following reading) the double value of the negative control. This has prompted us to conduct experiments with pure isolates of PVY (PVY^N and PVY^0) testing them by using IgG and EIgG for all PVY strains, only for the necrotic strain PVY^N and for PVA.

Material and Methods

Investigations of the serological relatedness of potato viruses Y and A were carried out initially in 5 experiments. Each experiment represented a series of simultaneous tests of potato plant leaves for the presence of PVY and PVA. A total of 879 plants have been screened in 5 tests, as follows: 638 samples in test 1, 25 samples of cv. Desiree in test 2, and in the remaining three tests 56, 82 and 78 plants of different potato cultivars and hybrids. The experiments presented and analyzed only those plants in which other potato viruses have not been detected (PVM, PLRV, PVS and PVX).

Each plant tested represented 1 sample, and all samples for testing were prepared in the same way. Potato leaves were homogenized in an extraction buffer in the approximate ratio of 1:20 (w:v). Each sample was tested by ELISA for the presence of PVY and PVA. A PBS buffer supplemented with 20 g/l polyvinylpyrrolidone and 0.5 ml/l Tween-20 was used for the extraction. For the identification of PVY, polyclonal antibodies (IgG and alkaline phosphatase conjugated IgG /EIgG/) were used, and for the identification of PVA and PVY^N monoclonal antibodies (IgG and alkaline phosphatase conjugated IgG) obtained from BIOREBA, Switzerland.

The extinction value E_{405} was read in a photometer 60 minutes after incubation from the addition of a substrate buffer with 0.75 mg/l substrate.

Following these analyses, an experiment was conducted as the previous one, with the difference that tests included the potato plants with pure isolates of potato viruses Y^N and Y^0. Reading of the reaction was done after 30, 60 and 120 minutes, 4.5, 48 and 62 hours.

Results and Discussion

The results from studies on the serological relatedness of PVY and PVA are presented in Table 1. In experiment 1, neither PVY nor PVA have been detected in any of 479 plants, i.e. the extinction value E_{405} was at the level of the negative control in using IgG and EIgG for PVY and IgG and EIgG for PVA. However, in 129 samples in which PVY was detected and the extinction value

averaged 1.450, all these samples (in 100% cases) , when IgG and EIgG for PVA were used, showed an increase in extinction, with the average value of 0.165. Of 10 samples with the presence of PVY (E_{405} averaging 1.602) all were infected with PVA, or, by using IgG and EIgG for PVA, the E_{405} value averaged 1.220.

Tab. 1. Extinction (E_{405}) values obtained by testing potato plants infected with PVY for the presence of PVY and PVA, i.e. using in the test IgG and EIgG* for PVY and PVA

Exp.	No. of potato plants tested	Average E_{405} value obtained using IgG and EIgG for PVY	Average E_{405} value obtained using IgG and EIgG for PVA
1	129	1.450 (+)	0.165 (?)
	10	1.602 (+)	1.220 (+)
	479	0.086 (-)	0.075 (-)
	Negative control (10 plants)	0.085 (-)	0.077 (-)
2	12	2.441 (+)	0.139 (?)
	10	0.077 (-)	0.080 (-)
	Negative control (2 plants)	0.078 (-)	0.079 (-)
3	26	1.434 (+)	0.172 (?)
	5	1.481 (+)	1.450 (+)
	14	0.080 (-)	0.079 (-)
	Negative control (1 plant)	0.080 (-)	0.078 (-)
4	43	1.477 (+)	0.156 (?)
	7	0.987 (+)	0.537 (+)
	30	0.075 (-)	0.079 (-)
	Negative control (2 plants)	0.075 (-)	0.080 (-)
5	42	0.634(+)	0.120(?)
	3	0.630(+)	1.058(+)
	29	0.081(-)	0.079 (-)
	Negative control (2 plants)	0.079 (-)	0.074 (-)
Σ	252	(+)	(?)
	24	(+)	(+)
	562	(-)	(-)
	1	(-)	(+)
	0	(+)	(-)
	Negative control (17 plants)	(-)	(-)

*conjugated to IgG alkaline phosphatase

Also, in experiment 2, of 12 samples with the assessed presence of PVY, in all of them in tests for PVA the E_{405} value was increased and after 60 minutes it averaged 0.139. In 10 samples in which PVY was not detected and which had average E_{405} values at the level of the negative control, the E_{405} value for PVA was at the level of the negative control (0.080). The same regularity of the relation of E_{405} values for PVY and PVA was recorded in experiments 3,4 and 5. The samples in which PVY was not detected did not show an increase in E_{405} value when using IgG and EIgG for PVA (Tab.1).

The analysis of the results of investigation presented in Table 2 revealed the regularity identical to that in the previous experiments. Tests of the isolates Y-2, Y-6 and Y-10 which belong to the Y^N strain, using IgG and EIgG for all PVY strains have shown the E_{405} values to be positive at all times of reading of the reaction, starting from 30 minutes. The same holds true for using IgG and EIgG for proving only Y^N, only with E_{405} values being considerably higher. However, by testing those isolates using IgG and EIgG for PVA, the E_{405} value after 30 minutes was 0.112 and the negative control 0.080, after 60 minutes 0.150 and the negative control 0.082, after 4.5 hours it was 0.376 and negative control 0.095 and, finally, after 48 hours it was 1.853 and the negative control 0.208. This is indicative of a certain degree of serological relatedness of PVY to PVA.

Tab. 2. Extinction (E_{405}) values obtained by testing potato plants infected with PVY^N and PVY^0 for the presence of PVY and PVA, i.e. by using in the test IgG and EIgG for PVY and PVA

Isolates of the strain (average)	Reading of the reaction after	E L I S A (E_{405}) IgG EIgG PVY*	IgG EIgG PVY^N**	IgG EIgG PVA
Y-2; Y-6; Y-10 (Y^N)	30 min	0.707	1.561	0.112
	60 min	1.425	2.409	0.150
	4.5 hrs	2.895	2.947	0.376
	48 hrs	3.009	3.053	1.853
Y-5; Y-11 (Y^o)	30 min	0.459	0.084	0.507
	60 min	0.933	0.087	0.948
	4.5 hrs	2.283	0.110	2.424
	48 hrs	2.944	0.319	2.819
Negative control (healthy tobacco and potato)	30 min	0.088	0.083	0.080
	60 min	0.095	0.084	0.082
	4.5 hrs	0.139	0.102	0.095
	48 hrs	0.519	0.254	0.208
Healthy Samsun	60 min	0.081	0.075	0.079
Average of 35 potato plants infected only with PVY^N	60 min	0.634	2.143	0.120
	120 min	1.040	2.581	0.162
	64 hrs			1.368
Average of 29 helthy potato plants	60 min	0.081	0.075	0.079
	120 min	0.089	0.076	0.085
	64 hrs			0.255
Pure pufer	60 min	0.069	0.068	0.069
	120 min	0.071	0.070	0.072
	64 hrs			0.148

* IgG and EIgG for proving all the strains of PVY
** IgG and EIgG for proving only the necrotic strain of PVY

Testing of the isolates Y-5 and Y-11 which belong to potato virus Y^0 by using IgG and EIgG for PVY has shown that at all times of reading the reaction

was positive and approximately at the same level as with the use of IgG and EIgG for PVA, whereas when using IgG and EIgG the reaction was negative only for Y^N. The current research failed to assess whether in this case the reaction was positive because PVA was also present or this strain of PVY was serologically more closely related to PVA.

Fig.1. The change of reaction values (extinction values E_{405}) in the function of time when testing the PVY isolates (PVY-2, PVY-6 and PVY-10) by using IgG and EIgG for PVY and PVA. (+) - plant material infected with PVY; (-) - healthy plants (negative control)

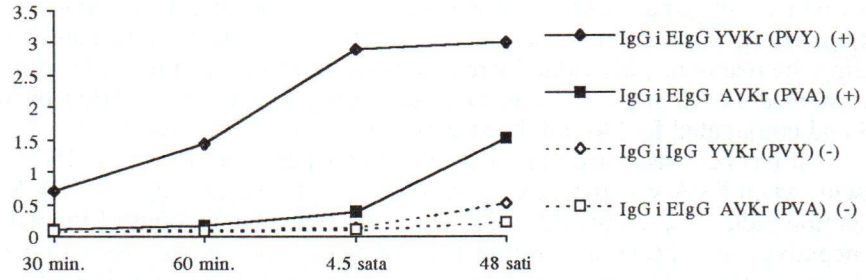
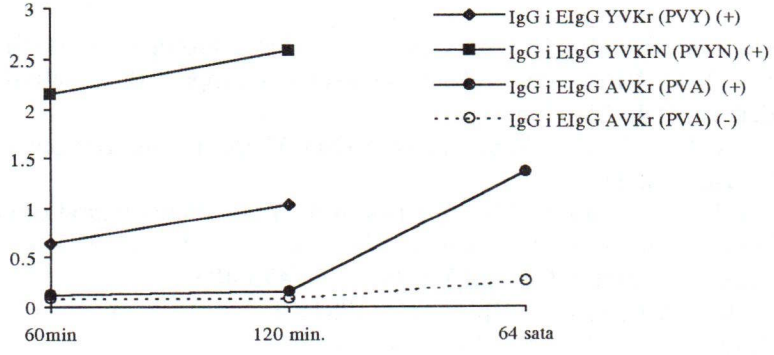


Fig.2. The change of reaction values (extinction values E_{405}) in the function of time whentesting plants infected with PVY^N by using IgG and EIgG for PVY, PVY^N and PVA. (+) -plant material infected with PVY; (-) - healthy plants (negative control)



De Bokx and Huttinga (1981), Hollings and Brunt (1981a), Hollings and Brunt (1981b), Bartels (1971) and Jones and Fribourg (1986) have reported, by citing other authors, the existence of serological relatedness of PVY to PVA. Fribourg and Nakashima (1984) pointed out to the serological relatedness between potato virus V (PVV) , potato virus A (PVA) and the strains

of potato virus Y (PVY). Harrison (1971) (cited after Rich, 1983) has also reported the existence of serological relatedness between PVY and PVA.

The results of these experiments indicate that it is possible, by using ELISA, to assess serological relatedness of potato viruses Y and A, which belong to the same potyvirus group.

Conclusions

By testing potato plants of different origin, it was found out that in the cases when only potato virus Y was present in the sample, with using IgG and conjugated IgG for potato virus A, the extinction value E_{405} increased when reading the reaction. This value increases in the function of time. Following this, tests were done on some PVY isolates belonging to the PVY^N strain by using IgG and conjugated IgG for all the strains of PVY, for PVY^N and PVA.

It has been assessed that in 100% cases where only PVY, i.e. PVY^N was present, when PVA was diagnosed, with the use of IgG and EIgG for PVA , the extinction value E_{405} increased. This value was twice the amount of the value of the negative control 60 minutes after reading of the reaction, and increased in the function of time. The phenomenon was not recorded when using the same antibodies in the samples in which PVY was absent.

These results indicate the existence of serological relatedness of PVY to PVA.

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NEKA ISKUSTVA U IDENTIFIKACIJI "*POTY*" VIRUSA KROMPIRA ELISA TESTOM

-originalni naučni rad-

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Rezime

U radu je vršeno proučavanje odnosa Y i A virusa krompira, odnosno seroploške srodnosti ova dva virusa na osnovu vrijednosti ekstinkcije (E_{405}) u ELISA testu.

Testiranjem biljaka krompira različitog porijekla, utvrđeno je da tamo gdje je u uzorku prisutan samo Y virus krompira (potato virus Y), kod korišćenja IgG i konjugovanog IgG za A virus krompira (potato virus A) dolazi do povećanja vrijednosti E_{405} kod očitavanja reakcije. Ta vrijednost se povećava u funkciji vremena. Posle toga vršeno je testiranje nekih izolata Y-VKr koji pripadaju soju $Y\text{-}VKr^N$ korišćenjem IgG i konjugovanog IgG za sve sojeve Y-VKr, za $Y\text{-}VKr^N$ i A-VKr.

Utvrđeno je da je u 100% slučajeva gdje je prisutan samo Y-VKr odnosno $Y\text{-}VKr^N$ kod identifikacije A-VKr odnosno kod korišćenja IgG i EIgG za A-VKr dolazi do povišenja vrijednosti ekstinkcije E_{405}. Ta vrijrdnost je u dvostrukom iznosu vrijednosti negativne kontrole nakon 60 minuta od očitavanja reakcije, a u funkciji vremena se povećava. Ova pojava nije zabilježena kod korišćenja istih antitijela kod uzoraka gdje nije prisutan Y-VKr.

Ovi rezultati ukazuju na postojanje serološke srodnosti između Y-VKr i A-VKr.