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The effect of phosphate buffer and temperature shock treatment on reaction of 'Red Kidney' bean primary leaves to inoculation with potato virus M

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Abstract

The influence of various factors on 'Red Kidney' bean reaction to inoculation with PVM was studied. The highest increase in the number of local lesions on inoculated primary leaves was obtained when inoculum was prepared in 0.066 M phosphate buffer pH 7.5. A significant but not consistent increase in the number of local lesions was obtained when inoculated leaves were rinsed with phosphate buffer instead of water or when the plants were submitted to thermal shock treatment 2-6 days after inoculation. Quick-drying of inoculated leaves instead of rinsing caused some increase of the number of local lesions but was difficult to perform. Rinsing inoculated leaves with phosphate buffer and temperature shock treatment did not improve the detectability of PVM by bioassay on 'Red Kidney' bean plants when highly diluted inocula or mild virus isolates were used as well as when the presence of PVM was assayed in plants resistant to virus infection.

INTRODUCTION

Phaseolus vulgaris L. cv. 'Red Kidney' is a good indicator plant for potato virus M (PVM) because it reacts with conspicuous local lesions upon inoculation of primary leaves (H i r u k i, 1970). Intact plants with primary leaves or detached primary leaves were efficiently used for inoculation and the virus was detected in leaves, tubers, eyes, and sprouts of potato plants (H i r u k i, 1973). Several factors have been found to affect the reaction of 'Red Kidney' bean primary leaves to inoculation with PVM (H i r u k i et al., 1974; D z i e w o ń s k a and O s t r o w s k a, 1973; K o w a l s k a and S k r z e c z k o w s k a, 1976). We tried to improve the detectability of PVM on 'Red Kidney' bean primary leaves by using some methods which have been shown to be effective for some other plant-virus combinations, namely: rinsing inoculated

leaves with phosphate buffer (Y a r w o o d, 1962), quick-drying of inoculated leaves (Y a r w o o d, 1963, 1973), and temperature shock treatment (Y a r w o o d, 1958; H a r r i s o n and J o n e s, 1971; J o n e s, 1973; F o s t e r and R o s s, 1975a, b; H e n d e r s o n and C o o p e r, 1977).

MATERIALS AND METHODS

Two severe (M55 and M24) and one mild (M57) PVM isolates described by K o w a l s k a (1978) were used throughout the experiments. They were maintained in greenhouse conditions on potato plants cv. 'Uran' and on tomato plants cv. 'Najwcześniejszy'. Inocula were prepared by grinding the infected leaves of these plants in a mortar in the presence of phosphate buffer pH 7.5. The primary leaves of intact 'Red Kidney' bean plants previously dusted with carborundum 400 mesh were inoculated when almost fully expanded (10-14 days after sowing). In each experiment 8-10 leaves or halves of the leaves were inoculated for one experimental treatment. The lesions were counted 9-10 days after inoculation. For statistical purposes the obtained lesion numbers were transformed according to K l e c z k o w s k i (1955). Each experiment was repeated several times and representative results were chosen for presentation. The experiments were conducted in uncontrolled greenhouse conditions with the temperature ranging from 20 to 30°C. The temperature shock treatments were performed by inoculating 'Red Kidney' bean plants in a cooled chamber (temperature 10-15°C) and transferring them 1-6 days later to greenhouse conditions. Quick-drying of inoculated leaves was performed with a hair dryer using th cold-air stream until no free water was visible on the leaf surface.

In experiments on the influence of rinsing of inoculated 'Red Kidney' leaves with 0.066 M phosphate buffer pH 7.5 on the detectability of PVM in susceptible (presumably high virus content) and resistant (low virus content) potato plants, the plants of potato cv. 'Certa' and plants of dihaploid *Solanum tuberosum* T_{22} (susceptible) or plants of the hybrids *S. gourlayi* × *S. tuberosum* 77-g-14a/15 and 77-g-14a/19 (resistant) were used as inoculum sources. These plants were inoculated with M55 isolate 4-6 weeks earlier.

RESULTS

Influence of pH of inoculum

When the inoculum was prepared by grinding infected leaves in the presence of phosphate buffers of different pH levels, the highest number of local lesions was obtained on 'Red Kidney' bean primary leaves inoculated with inoculum prepared in pH 7.5 buffer (Fig. 1). Significantly lower lesion counts were obtained

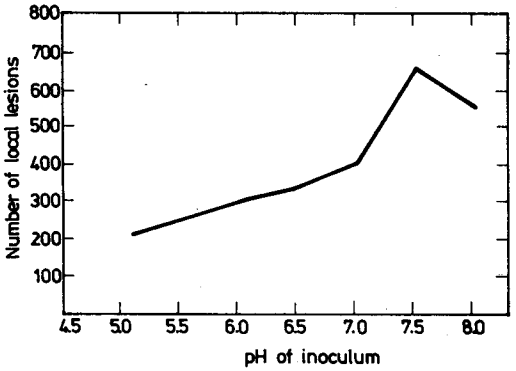

Fig. 1. The influence of pH of inoculum on the infectivity of PVM (isolate M55) to 'Red Kidney' bean primary leaves

when buffers of lower pH were used. PVM inoculum in phosphate buffer pH 7.5 was therefore used in most of the further experiments.

Rinsing or drying 'Red Kidney' bean leaves

The highest number of local lesions was obtained on leaves which were rinsed with phosphate buffer pH 7.5 after inoculation (Fig. 2). The number of lesions obtained on leaves dried after inoculation was slightly lower, but still significantly higher than on leaves rinsed with water. Drying leaves after inoculation took 40-60 s per leaf. Attempts to shorten this time by using a warm-air stream did not improve the results; attempts to use a high pressure air stream were unsuccessful because the leaves were seriously damaged.

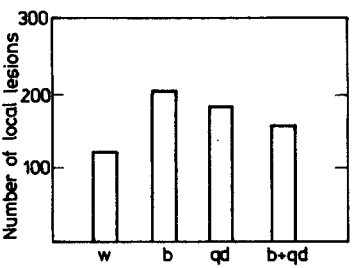

Fig. 2. The influence of rinsing of 'Red Kidney' bean primary leaves with water (w) or phosphate buffer (b) and the influence of quick-drying of these leaves (qd) on the number of PVM (isolate M55) local lesions

Rinsing inoculated leaves with phosphate buffer and detectability of PVM

The use of phosphate buffer for rinsing leaves inoculated with decreasing virus concentrations did not improve the detectability of PVM when compared with tap water (Fig. 3). In many cases rinsing with water gave even slightly better results. Local lesions were still obtained on leaves inoculated with sap diluted to 10^{-3} independent of the method of rinsing.

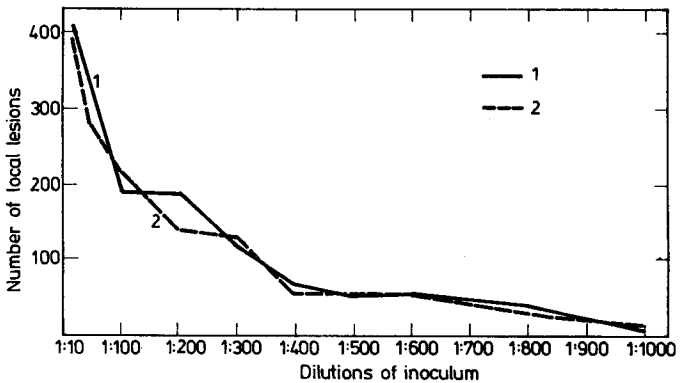

Fig. 3. The detectability of PVM (isolate M55) in different concentrations by bioassay on 'Red Kidney' bean primary leaves rinsed with water (1) or phosphate buffer (2) after inoculation

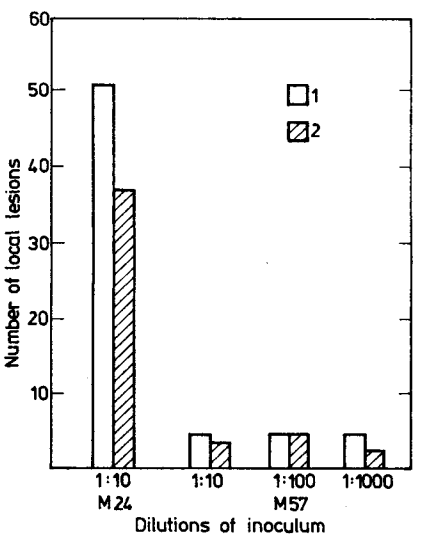

Fig. 4. The detectability of severe (M24) and mild (M57) isolates of PVM by bioassay on 'Red Kidney' bean primary leaves rinsed with water or phosphate buffer after inoculation. 1 – leaves rinsed with buffer; 2 – leaves rinsed with water

Similar results were obtained when phosphate buffer was used to improve the detectability of the mild PVM isolate (Fig. 4). Much higher numbers of lesions were obtained on leaves inoculated with severe virus isolate but mild isolate could be still detected independent of the sap dilution used for inoculation and independent of the method of rinsing.

No improvement of PVM detectability was also observed by rinsing leaves with phosphate buffer when potato plants with resistance to PVM from *S. gourlayi* Hawk. were used as inoculum source (Fig. 5). Inoculation of 'Red Kidney' bean leaves with sap from these plants infected with PVM produced much lower numbers of lesions than inoculation with sap from PVM susceptible potato plants. In every case, rinsing of inoculated leaves with phosphate buffer increased the lesion number when compared to rinsing with tap water, but this increase was not significant. Local lesions were obtained on leaves inoculated with sap from all PVM resistant potato plants independent of the method of rinsing.

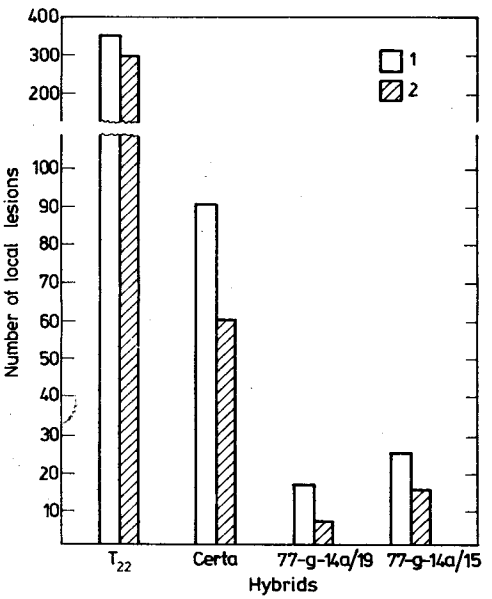

Fig. 5. The detectability of PVM (isolate M55) in susceptible (potato cv. 'Certa' and *S. tuberosum* dihaploid T_{22}) and resistant (*S. gourlayi* × *S. tuberosum* hybrids) potato plants by bioassay on 'Red Kidney' bean primary leaves rinsed with water or phosphate buffer after inoculation. 1 – leaves rinsed with buffer; 2 – leaves rinsed with water

Temperature shock treatments

The results of several trials are summarized in Table 1. Transferring inoculated plants from the cooled chamber to the greenhouse 1 day after

inoculation did not influence the number of lesions obtained on inoculated primary leaves. On the other hand, when plants were transferred 2-6 days after inoculation, the number of local lesions increased and the maximal increase was obtained when plants were transferred on 3rd and 4th day after inoculation. The results were similar for plants rinsed with water and with phosphate buffer.

We were not able to demonstrate that temperature shock treatment improves the detectability of PVM on 'Red Kidney' bean when mild virus isolate and high

Table 1

The average number of local lesions on 'Red Kidney' bean primary leaves inoculated with PVM (isolate M55) in the cooled chamber and rinsed with tap water (w) or with phosphate buffer (b), and then transferred to the greenhouse 1-3 days after inoculation

Trial No.	Plants inoculated in the greenhouse and not transferred		Plants inoculated in the cooled chamber and transferred to the greenhouse after:					
			1 day		2days		3 days	
	w	b	w	b	w	b	w	b
1	17	–	–	–	–	–	99	–
2	11	–	20	–	35	–	85	–
3	1	1	–	–	–	–	12	7
4	50	34	–	–	–	–	138	72
5	87	93	–	–	–	–	172	194
6	73	–	–	–	–	–	95	–

Table 2

The influence of temperature shock treatment on the number of local lesions produced by mild (M57) and severe (M55) PVM isolates on 'Red Kidney' bean primary leaves. Plants treated with thermal shock were transferred 4 days after inoculation from the cooled chamber (15°C) to the greenhouse (20-25°C)

Dilution of sap	Average number of local lesions on 8 leaves			
	plants in the greenhouse, no shock treatment		plants treated with thermal shock	
	M57	M55	M57	M55
1:10	370	391	78	322
1:100	132	246	31	140
1:300	32	62	12	57
1:500	26	38	9	46
1:800	13	34	5	32
1:1000	9	20	3	32

sap dilutions were used for inoculations (Table 2). In fact the number of lesions obtained was even lower in the plants which were transferred from the cooled chamber to the greenhouse than on leaves of plants which were kept in the greenhouse from the day of inoculation untill the day of counting lesions.

DISCUSSION

The results presented here confirm the opinion that the 'Red Kidney' bean is a very good indicator plant for PVM bioassaying (Hiruki, 1970, 1973; Hiruki et al., 1974). Inoculated primary leaves of this plant reacted consistently with conspicuous local lesions to inoculation with different virus isolates used in low concentrations and obtained from different plants. Using several methods which were expected to enhance the reaction of inoculated plants we succeeded in obtaining higher numbers of local lesions on inoculated leaves but in no case could we obtain the reaction of plants which would not react when inoculated and treated by "conventional" methods.

Phosphate buffer pH 7.5 proved to be the best medium for preparing PVM inoculum and confirmed previous data (Hiruki et al., 1974; Kowalska and Skrzeczkowska, 1976) and the general opinion that slightly alkaline buffers are better for preparing virus inocula than slightly acidic ones (Fulton, 1964).

Among several methods tested, temperature shock treatment was the most effective and consistent in increasing the number of lesions upon inoculation with PVM. This effect is probably due to the increased susceptibility of some cells in which a high rate of virus multiplication takes place in response to change of temperature (Harrison and Jones, 1971; Foster and Ross, 1975a). On the other hand no visible lesions could be obtained on the leaves of potato seedlings inoculated with PVM and heated for 3-60 s by dipping in hot (50-70°C) water between the 3rd to 8th day after inoculation nor on leaves inoculated with PVM and stained according to the method described by Lindner et al. (1974) with iodine 3-17 days after inoculation to produce starch lesions (unpublished results of the senior author). These results indicate that some methods of obtaining local lesions may be specific for some plant-virus combinations.

. Quick-drying proved to be less efficient method of increasing the number of local lesions on 'Red Kidney' bean primary leaves inoculated with PVM. This result is not contradictory to Yarwood's opinion (1963, 1973) that quick-drying is the best technique of increasing the number of local lesions produced by different viruses on leaves or cotyledons of several test plants. PVM was not included in Yarwood's experiments nor was the 'Red Kidney' bean. Moreover, in Yarwood's experiments the residues of inoculum were blown off the inoculated leaves and drying was completed within 4 s whereas in our experiments the leaves

were dried rather by accelerated evaporation of water and 40 to 60 s were needed to complete the drying. This difference could greatly influence the results since the advantage of the quick-drying procedure is that it quickly removes the residues of plant sap used for inoculation from inoculated leaves and this way it precludes some deletarious effects of these residues on infection sites (Yarwood, 1963). We attempted to use some other equipement for quick-drying but the high pressure air stream always caused serious damage of dried leaves. In addition, we used inoculum prepared in phosphate buffer and Yarwood (1963) has found that the quick-drying procedure was the most efficient when inoculum was prepared in water. Irrespectively of the technique used for drying of inoculated leaves this method is too laborious to be used for standard inoculations. This opinion is strongly supported by the fact that the time is long past when first, very encouraging results of using this technique for enhancing virus infections were published and still rinsing inoculated leaves is routinely used.

Rinsing inoculated leaves with phosphate buffer instead of water increased the number of local lesions on primary leaves of 'Red Kidney' bean plants in many instances but the results were not consistent. This inconsistency and that of the effects of temperature shock treatment were probably due to the influence of other factors (e.g. virus source, the condition of test plants, light, temperature etc.) on reaction of 'Red Kidney' plants to inoculation with PVM. The experiments were conducted in uncontrolled and not reproducible greenhouse conditions. In no case did rinsing inoculated 'Red Kidney' primary leaves with phosphate buffer nor treating plants with temperature shock prove to be of practical value. None of these two methods improved PVM detectability when inoculum of low virus content was used nor when 'Red Kidney' bean was inoculated with mild PVM isolate or when plants in which PVM is difficult to detect were used as virus sources. It is very likely that in all these three cases we were dealing with the same main factor limiting the detectability of the virus. It is often postulated that mild strains of viruses are the slowly replicating strains which do not attain high concentrations in plant tissues. Similarly, the resistance of *Solanum gourlayi* to PVM may be caused by inhibition of virus multiplication which results in low virus content in the tissues of plants carrying this resistance (Waś et al., 1980). Thus, it is still possible that rinsing inoculated leaves with phosphate buffer may be helpful when some other factors limiting infection would operate (e.g. virus inhibitors).

In some experiments, when the incubation temperature of inoculated plants exceeded 25°C we were not able to obtain local lesions on 'Red Kidney' bean primary leaves inoculated with PVM which confirms the results of some previous works (Dziewońska and Ostrowska, 1973; Hiruki et al., 1974; Kowalska and Waś, 1976). This seems to be almost the only factor limiting the usefulness of this test plant for PVM bioassaying. It is also

worth remembering that in spite of H i r u k i's (1970) report 'Red Kidney' bean is not immune to potato virus S (K o w a l s k a, 1977).

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Wpływ buforu fosforanowego i szoku termicznego na reakcję pierwotnych liści fasoli 'Red Kidney' na inokulację wirusem M ziemniaka

S t r e s z c z e n i e

Badano wpływ różnych czynników na reakcję roślin fasoli 'Red Kidney' na inokulację wirusem M ziemniaka (PVM). Największą liczbę plamek lokalnych na inokulowanych pierwotnych liściach fasoli uzyskano, kiedy inokulum PVM przygotowano w 0,066 M buforze fosforanowym o pH 7,5. Istotne, choć nie we wszystkich doświadczeniach, zwiększenie liczby plamek uzyskiwano, kiedy liście po inokulacji płukane były takim samym buforem zamiast wodą lub kiedy rośliny poddawano szokowi termicznemu w 2-6 dni po inokulacji. Suszenie liści po inokulacji w miejsce płukania powodowało nieco mniejszy przyrost liczby plamek i było zabiegiem trudnym do wykonania. Ani płukanie liści buforem fosforanowym, ani poddawanie liści szokowi termicznemu po inokulacji nie zwiększyło wykrywalności PVM testem biologicznym na fasoli 'Red Kidney', kiedy stosowano bardzo rozcieńczone inokula, inokulowano rośliny słabym izolatem wirusa lub badano obecność wirusa w roślinach odpornych na porażenie.