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EFFECT OF ANTIVIRAL CHEMICALS ON PRODUCTION OF VIRUS X FREE POTATO TUBERS

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Abstract

Growing tips, nodal segments with rudimentary buds and sprout discs excised from PVX infected tubers and seedlings were grown in modified and enriched medium. Thiouracil and malachite green were used as antiviral chemicals in different concentrations. Thiouracil was found to be more effective and better than malachite green for antiviral properties. Chemicals either arrested virus multiplication or rendered it inactive. A combination of the antiviral chemicals incorporated in medium did not result in synergistic action as presumed. It was further observed that potato extract did not support root initiation or development, as claimed by Manzer (1958) and others.

Introduction

Due to its inherent stability under varied environs, potato virus X is generally present in all parts of the world where potatoes are grown. Potato virus X may cause very severe damage if tobacco mosaic virus or potato virus Y attacks the crop as a second virus (Dr. F.A. Ross, Personal communication). Majority of potato varieties are susceptible. The disease may pass from generation to generation undetected and the ultimate result is the degeneration of cultivars.

The presence of virus X in the tubers has never been challenged. The author found the virus in sprouts, leaves and tubers. The local lesions on test plant leaves were least from tuber juice, maximum from sprouts and intermediary from stem and leaf sap of infected plants.

Thiouracil and malachite-green have been used as antiviral chemicals with positive and negative results. Takahashi (1948, 1957) reported inhibition of tobacco mosaic virus with malachite green. Nagayoshi & Livingston (1958) and Norris (1954) obtained potato seedlings free of virus X by treating shoot tips with malachite green in tissue culture. Thompson (1956) on the other hand, failed to get virus X free seedlings after using malachite green. Bawden *et. al.*, (1948), Manzer (1958), and Nagayoshi & Livingston (1961), produced PVX free potato seedlings by using thiouracil at different concentrations either by incorporating the chemical in growing medium or by dip treatment.

Keeping in view the economic importance of PVX, impracticability of controlling it by chemical spray in the infected fields, and conflicting results of antiviral chemicals, it was considered necessary to reasses the antiviral activities of thiouracil and malachite green, using different types of tissues and various concentrations of the chemicals to obtain PVX free seed potato stock.

Materials and Methods

White's (1943), modified solid medium was prepared from which four components, viz., glycine, thiamine, pyridoxine and nicotinic acid were deleted. Instead, potato extract at the rate 500 ml. per litre of medium was added (Manzer, 1958, Reichle, *et. al.*, 1960). Potato extract was prepared by boiling 200 grams of diced potato in 500 ml. of distilled water and filtered through cheese cloth. Sucrose and agar were dissolved separately and mixed with potato extract. Immediately after, chemicals were added and dissolved, thiouracil or malachite green were incorporated according to schedule.

As potato tubers infected with virus X were freshly harvested, dormancy was broken by treating them with Rindite for three days. The tubers were kept in gas tight containers and Rindite was administered 1/3 of the dose each day. Due to this treatment the eyes started swelling after seven days. Tubers were stored at room temperature in diffused daylight for development of green sprouts and later planted in pots. To ascertain the presence of virus X in the tubers, sap from sprout and seedlings was inoculated on test plant (*Gomphrena globosa L.*) leaves. For inoculation carborundum-Cheese-cloth-pad method was used.

Three types of tissues, (1) growing tips (3-4mm) (2) nodal segments from young seedlings (5 x 8 mm) and (3) sprout (2 x 5 mm) were used in this investigation. Tissues were transferred into test tubes containing 5 ppm and 10 ppm thiouracial or 10 and 15 ppm malachite green incorporated media. In the second treatment the tissues were soaked for two hours in solutions of thiouracil or malachite green at the same concentrations and transferred to the slants having plain medium. In the third treatment 10 ppm, of each thiouracil and malachite green were combined and incorporated in the medium to receive different types of tissues. Controls were grown in the medium without any antiviral chemicals. The test tubes were incubated at $72 \pm 2°F$ in continuous light. The tissues were surface sterilized with 20% clorax before they were transferred into the medium. To check microbial growth 1000 ppm streptomycin sulphate was added. Fortification of the medium was done by addition of 100 ppm gibberellic acid. After five weeks indole-acetic-acid (IAA) at the rate of 1 ppm was also applied to each slant with the help of hypodermic syringe.

All the rooted seedlings were transplanted to 6″ pots filled with sterilized vermiculite. After the seedlings were fairly developed, juice extract from leaves of control and treated ones was inoculated on the leaves of *Gomphrena globosa* L. (8 leaves stage) for observation.

Results and Discussion

First sign of growth was visible in the axillary buds of the nodal segments after seven days of incubation. Within a period of three weeks the length of the emerging shoots was approx. 1.5 cm and the leaf lamina was typically lanceolate. The tissue from the growing tips showed similar growth pattern, but there was marked variation in the length of the shoots. The variation may be attributed to the ontogeny and state of development of the tissues at the time they were transferred to the media. Undoubtedly gibberellic acid might have also played its role rather than the antiviral chemicals because similar growth pattern was observed in tubes where antiviral chemicals

were not added. Although, the tissues were taking up growth, there were no signs of root emergence. The sprout discs presented a different picture. Practically all of them, control or treated, sent out small rootlets from different loci in the peripheral region but none of them produced shoots even after five weeks.

At the very out-set no hormone was applied to the medium, as reported by Manzer (1958) and others that potato extract stimulated and supported growth of both root and shoot. But this contention could not be confirmed under the present experimental conditions. The emergence of only small pegs from the discs, could hardly be taken as a criterion of the effect of potato extract. Those small rootlets failed to develop what could be termed as root system. The application of IAA, after five weeks, did show some effect. Roots started initiating and developing in all type of tissues excepting in those where no IAA was added. It took only 3-4-weeks to develop a real root system. The number of survivors was least in case of malachite green and thiouracil combined treatment for which no explanation could be offered at this stage. The number of survivors in the control and treated materials were not materially different, thus giving an indication that antiviral chemicals did not exert any deleterious effect on growth and development. However, the heavy mortatily of the tissues could be due to some undetermined factors.

All the surviving seedlings from thiouracil treatment either incorporated or dip, appeared to be virus X free because the leaf extract from the seedlings, when inoculated on *Gompherna globosa* leaves, did not produce local lesions, whereas sap from control seedlings did. Malachite green showed erratic behaviour irrespective of the method of application. All the seedlings from sprout discs carried virus X. Some seedlings from nodal segments and growing tips were disease free and others diseased (Table I).

TABLE I. Effect of thiouracil and malachite green on different types of potato tissues.

Type of Tissues	Control	MALACHITE GREEN				THIOURACIL				Malachite Green and Thiouracil incorporated in medium
		Incorporated in medium		Dip Treatment		Incorporated in medium		Dip Treatment		
		10 ppm	15 ppm	10 ppm	15 ppm	5 ppm	10 ppm	5 ppm	10 ppm	10 each chem
Nodal Segment	9/3+	9/2+	9/3—	9/4+	9/2—	9/4—	9/5—	9/3—	9/2—	9/2+
Growing tips	9/3+	9/4—	9/2—	9/3—	9/2+	9/4—	9/3—	9/5—	9/4—	9/2+
Sprout discs	9/4+	9/4+	9/2+	9/4+	9/3+	9/6—	9/3—	9/3—	9/2—	9/1+

\+ presence of potato virus X in seedlings,
— absence of potato virus X in seedlings.,
Numerator indicates original number of tissues used.
Denominator indicates the number of seedlings that survived.

It may be mentioned here that movement of potato virus X in plants is quite erratic and may pose some problem and confusion and cousequently one has to be very careful in screening. The results of these experiments are in conformity with those of Bawden & Kassanis (1958), Manzer (1958), Norris (1954) Nagayoshi & Livingston (1961), Takahashi (1948, 1957) and are in disagreement with that of Thompson (1956) only. It is also suggested from the results that antiviral chemicals could be used to have PVX free seed stock and thiouracil would comparatively be more effective than malachite green.

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