Cytological Studies of Broad Bean, *Vicia faba* L., Plants Infected with Pea Mosaic Virus

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ABSTRACT

Broad bean, *Vicia faba* L., seedlings grown in the greenhouse were used as test plants and as healthy controls. Inoculum consisted of a mild strain of pea mosaic virus. Stems and stripes of epidermal cells from healthy and diseased plants were sectioned and studied before and after fixation. Cells of the diseased tissues were usually smaller in size than those of the healthy ones. Infected phloem tissues were dense and stained dark-purple. Inclusion bodies were found in the cytoplasm and nuclei of the epidermal cells obtained from infected leaves and stems. The cytoplasmic inclusions were distinct, amorphous and granular in texture. Intranuclear crystalline inclusions were mainly present within the nucleoli. No inclusions of similar appearance were found in sections obtained from healthy tissues.

INTRODUCTION

The presence of viral inclusions in cells of infected tissue was first noted by Iwanowski (7). Bawden (2) and McWhorter (10) presented reviews of the literature on intracellular inclusions. Several review articles on the anatomy of plant virus infections and plant virus inclusions have appeared (3,4,12).

The objective of this study was to determine the cytological abnormalities that could occur in tissues of broad bean plants infected with a mild strain of pea mosaic virus (PMV).

MATERIALS AND METHODS

Samples of freshly harvested young leaves were obtained from broad bean plants infected with a mild strain of PMV (6). Inoculum was prepared by grinding the infected leaves in distilled water. Broad bean seedlings grown in the greenhouse were used as test plants and as healthy controls. Inoculations were made by rubbing carborundum dusted leaves of test plants with the expressed juice using the forefinger. Control plants were rubbed with distilled water.

Healthy and virus-infected stem samples of corresponding ages and degrees of maturity were sectioned and studied while fresh, or after fixation, and embedding in paraffin. Sections were made 15 μ thick then were stained with iron-hematoxylin (8). In addition, epidermal cells were stripped from healthy and infected leaves and stems, then were fixed, dehydrated and stained with Giemsa stain (1).

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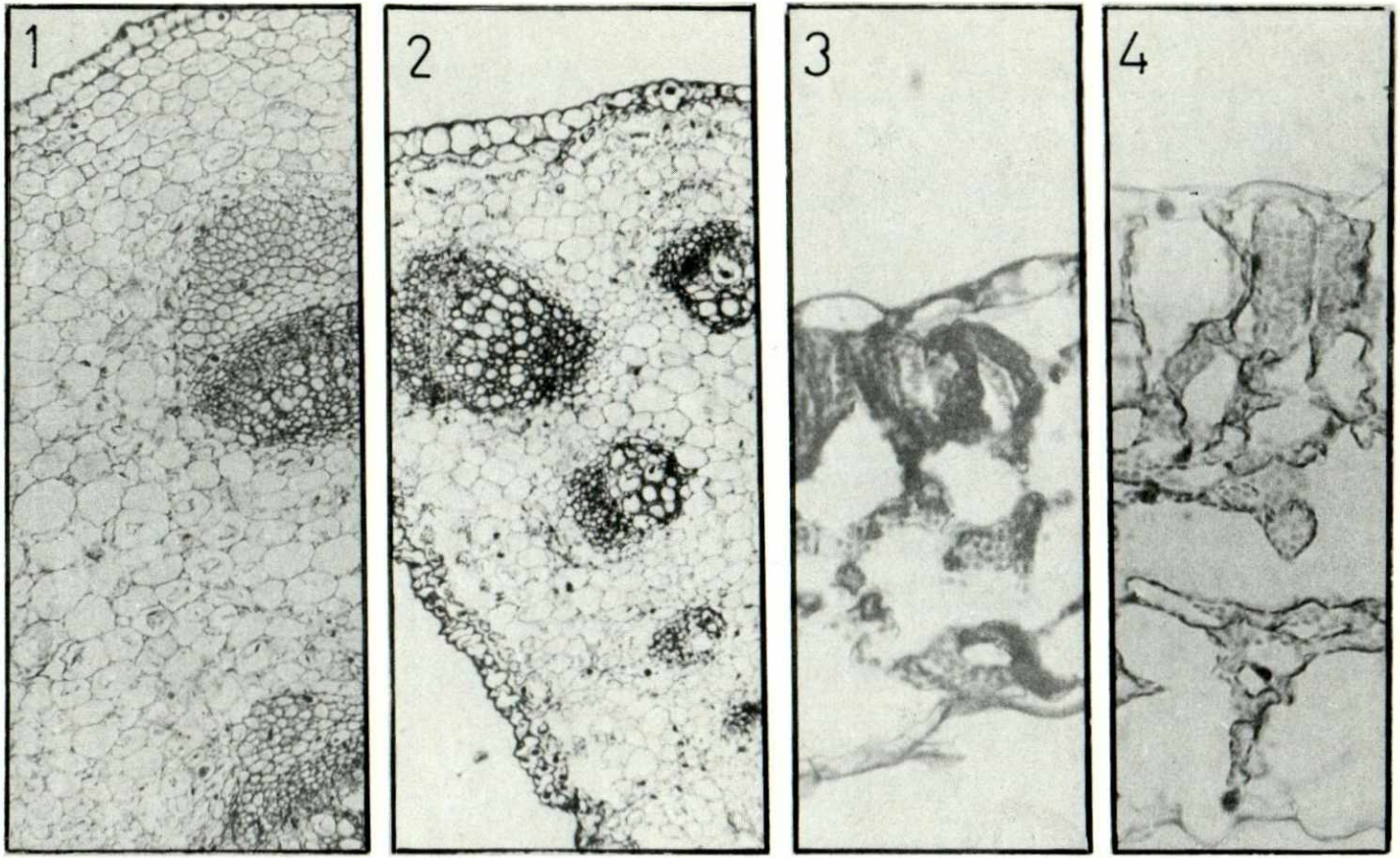

Fig. 1. (1) Cross section of stem from a healthy broad bean plant. The epidermis is one cell layer, and the cortex is formed from several layers of parenchyma cells. Phloem is formed from several, well-arranged layers of cells (× 300). (2) Cross section through stem from an infected plant with PMV. Note that cells of different tissues were usually smaller in size than were those present in healthy ones. Phloem tissue stained dark purple and appeared abnormally dense (× 300). (3) Cross section from infected leaf that showed external symptoms as dark green islands. Note that chloroplasts were found to be dense and numerous in the upper layers of palisade cells, but their distribution was normal in leaf tissues of healthy plants as in 4 (× 900).

RESULTS AND DISCUSSION

Microscopic examination of sections from healthy stems showed an epidermal layer and several layers of cortical parenchyma cells containing chloroplasts (Fig. 1(1)). In cross sections from systemically infected plants (Fig. 1(2)), the cortical tissue cells and vascular bundles were usually smaller in size than those of the healthy ones. Diseased xylem tissues were formed from rows of tracheary elements and the cells appeared structurally normal.

Phloem of healthy plants was formed from several, well-arranged layers of cells (Fig. 1(1)), but phloem of infected plants contained few layers (Fig. 1(2)). Several cells in the diseased phloem tissue stained dark-purple and appeared abnormally dense. Necrosis and death of cells of infected tissue was not noticed. It is known that the earliest pathological changes, for some viruses, occur in the phloem. For instance, in the case of potato leaf roll virus, the phloem of infected potato plants develops normally but is killed by the infection. Other viruses may lead to abnormal growth and differentiation of the phloem before necrosis occurs (5). However, in some viral diseases abnormal growth occurs, but it is not necessarily followed by necrosis (14).

In some infected leaves, external symptoms appeared as dark-green islands on the upper surface, and are not usually apparent when viewed from the underside. Upon microscopic examination of cross sections from these dark-green areas, chloroplasts were found to be dense and numerous in the upper layers of the palisade cells (Fig. 1(3)); whereas, in healthy plants, the distribution of the chloroplasts was normal in both the palisade and mesophyll tissues (Fig. 1(4)).

Epidermal cells of infected leaves and stems, stained with Giemsa stain, contained inclusion bodies in both the cytoplasm and the nuclei (Fig 2(2 and 3)). No inclusions of similar appearance were found in sections obtained from comparable healthy leaves or stems (Fig. 2(1)). The cytoplasmic inclusions (Fig. 2(2)) were distinct, with clearly defined borders and granular in texture. These inclusions also occurred frequently within the guard cells of stomata. Intranuclear inclusions were mainly present within the nucleoli (Fig. 2(3)). It was noticed that the inclusion bodies within the cytoplasm were of the amorphous type, but the intranuclear ones were of the crystalline type.

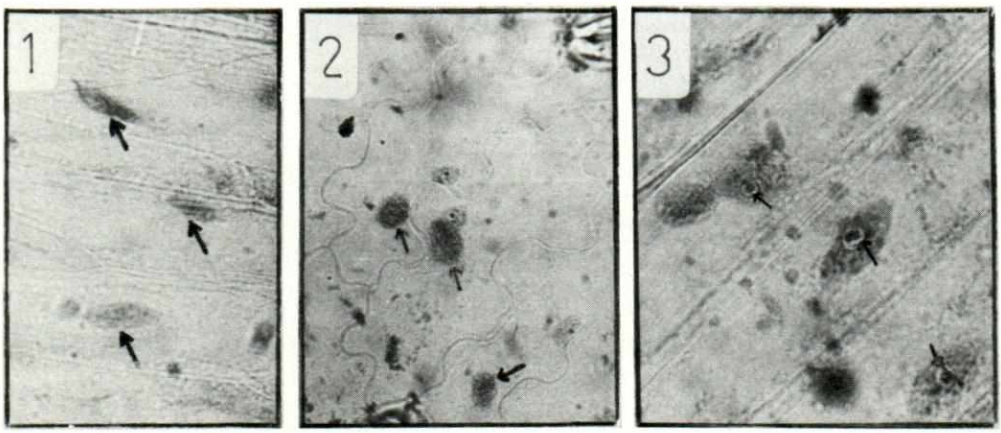

Fig. 2. (1) Epidermal cells of stem from a healthy broad bean plant. Nuclei (arrows) were normal and no inclusion bodies were found in them (× 1000). (2) and (3) Epidermal cells of leaves and stems obtained from a broad bean plant infected with PMV. Note that inclusion bodies were found in the cytoplasm (arrows) and nuclei. Intranuclear inclusions were mainly present within the nucleoli (arrows) as in (3) (× 1900).

Similar cytological changes were observed long ago by McWhorter (11) who found, in *V. faba* infected with Pisum virus 2 and Phaseolus virus 2, crystalline inclusion bodies in both the cytoplasm and nuclei of infected host cells. Mueller and Koenig (13) and Weintraub and Ragetli (16) found that crystalline inclusions of bean yellow mosaic virus frequently occurred in the nucleolus of infected *V. faba* cells. Intranuclear crystalline inclusions have been also seen by light microscopy in cells infected with severe etch virus in tobacco (9,15).

Cytological investigations of inclusions can be used to indicate relationships among many viruses, and in some cases for diagnosis (4). These uses will be expanded as additional studies add to the knowledge of inclusions' morphology, structure, and composition.

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دراسات تشريحية على نباتات الفول المصابة بالفيروس المسبب لمرض التبرقش بالبازلاء

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المستخلص

سبق وأن درست الخواص الطبيعية والبيلوجية للفيروس المسبب لمرض التبرقش فى نباتات الفول ووجد أنه هو الفيروس المسبب لمرض التبرقش فى البازلاء . وقد أجريت دراسات تشريحية باستعمال المجهر المركب على عدة قطاعات رقيقة من أنسجة سيقان نباتات الفول المصابة بالفيروس وأوضحت هذه الدراسة أن خلايا أنسجة البشرة والقشرة واللحاء المصابة كانت أصغر حجما وأقل عددا من خلايا الأنسجة السليمة ، كما أن خلايا أنسجة اللحاء المصاب ظهرت حبيبية الشكل .

كما أظهرت الدراسات التشريحية على وجود أنواع من الاجسام المحتواة (Inclusion bodies) داخل سيتوبلازم ونوايا خلايا البشرة المصابة ، وأن الاجسام المحتواة بالسيتوبلازم كانت واضحة ، حبيبية ، وأشكالها غير منتظمة ، بينما الاجسام المحتواة بالنوايا فكانت من النوع البلورى وموجودة عادة داخل النويات . وعند فحص مجموعة من قطاعات رقيقة من أنسجة نباتات فول غير مصابة اتضح عدم وجود الأجسام المحتواة داخل سيتوبلازم أو نوايا الخلايا .