Histogenesis of potato light sprouts in healthy plants and those infected with potato leaf roll virus

GRAŻYNA GARBACZEWSKA

Department of Botany, Institute of Plant Biology, Warsaw Agricultural University,
Rakowiecka 26/30, 02-528 Warszawa, Poland

(Received: October 8, 1979)

ABSTRACT

Developmental stages of potato (*Solanum tuberosum*) light sprouts in healthy plants and those infected with potato leaf roll virus (PLRV) have been distinguished. Potato light sprouts from healthy tubers differentiate in the early stages to the apical and basal parts, which are characterized by distinct dynamics of growth. Ultrastructural examination of apex meristem and phloem ontogeny was carried out for healthy plants. In the development of potato light sprouts of plants infected with PLRV a great many anomalies have been described. Three types of light sprouts developed from infected tubers. Anatomical investigations indicated a rapid growth of the number of necroses in the phloem, and submicroscopic observations showed the presence of virus particles and cytopathological structures: tubular and vesicular structures, paramural bodies and callose deposits. In the companion cells of the phloem the occurrence of very regular inclusions of virus particles was recorded.

Research on viral diseases of the potato requires a thorough knowledge of the structure of the healthy plant and of its organogenesis. Artschwager (1918a, 1924) carried out extensive studies on the anatomy and development of *Solanum tuberosum*. A number of later papers concerning this plant were undertaken for economic reasons and dealt above all with the physiological differences between healthy plants and those infected with pathogens. These studies were undertaken to develop diagnostic methods of disease detection.

One of the most dangerous potato diseases causing the greatest drop of yields is leaf roll. It is caused by the icosahedric virus of particle size 25 nm (Peters, 1967). This virus belongs to the group *Luteovirus* (Rochow and Israel, 1976) and is denoted by the symbol PLRV (potato leaf roll virus). The presence of this agent is the cause of patho-

logical changes in the above ground parts of the plant and affects also the underground organs serving for vegetative reproduction. It was found that PLRV causes a different type of tuber germination; it may completely inhibit the process of eye sprouting or change the course of development of the light sprouts (Baercke, 1968).

Observations in the electron microscope revealed that PLRV particles are localized in the phloem of the infected plant (Murayama and Kojima 1973; Golinowski, 1975, 1976; Garbaczewska, 1977, 1978; Hepp, 1976).

A review of the pertinent literature indicates the absence of descriptions of the developmental stages of light sprouts both in healthy and virus infected plants. The present investigations were undertaken to gain a better knowledge of the development of this type of *Solanum tuberosum* sprouts and of the histogenesis of the apical meristem tissues and phloem. It was also endeavoured to establish whether virus particles may be localized in apical meristem cells and whether there are changes in the ultrastructure of cells of this region.

MATERIAL AND METHODS

The material investigated consisted of eyes of potato tubers and light sprouts. The studies were performed on the following potato varieties: 'Apta', 'Bolko', 'Sieglinde', 'Uran' and 'Krokus'. These varieties were chosen from the point of view of the different reaction of their tuber eyes in germination.

Both healthy and PLRV-infected plants were used in the investigations. The plants were infected in the most effective way, that is by twofold placing of aphids on the plant leaves at the stage of 2-3 internodes. The tubers were pregerminated in January in appropriate temperature, humidity and light conditions.

Morphological observations were recorded from January to May and photographic documentation and diagrams were prepared. For microscopic examination tissues were taken at several dates, according to the developmental stage of the sprouts: on Jan. 20, Feb. 20, March 5, March 24, April 21. Whole light sprouts were taken for inspection in the light microscope, together with a fragment of the tuber or else they were divided into segments and fixed in FAA. After dehydration they were embedded in parafin. Sections 6 μ thick were cut on a Reichert rotating microtome. The sections were stained with: iron haematoxylin according to Heidenhain, complexely with lacmoid after Cheadle et al. (1953). Observations and documentation were recorded from a type Zeiss NU-2 light microscope.

For ultrastructural examination small tissue sections were taken

Plate 1

Morphology of light sprouts from healthy potatoes. The photographs show the development stages of the sprouts observed under conditions of pregermination from January (phot. A — eye in resting stage) to the end of March (phot. E and F). Light sprouts in stage of distinct differentiation to apical (a) and basal (b) part; in the strongly developed basal part adventitious roots and stolons are visible. $\times 2$

Plate 2

Diagrams of light sprouts from healthy potato plants prepared on the basis of anatomical studies of successive developmental stages. Distribution of meristematic tissues (double hatched) and vascular system (single-hatched) in light sprouts at various developmental stages: B₁ — resting eye stage, B₂ — sprout forming stage, B₃ — stage of elongation and sprout differentiation, B₄ — stage of dominance of basal part, B₅ — stage of dominance of apical part

Plate 3

Shoot apical meristem in light sprouts of healthy potato plant

A. Central longitudinal section through light sprout apex of Bolko variety. The sprout is in the stage of elongation and differentiation. In the promeristem two tunica layers can be distinguished (t) which differ structurally from the lower situated cells of the corpus (k). Peripheral meristem (p) is built of elongated cells with dense cytoplasm and differs from the central one (c) the cells of which are isodiametric, larger and more vacuolized. $\times 750$. B. Longitudinal section through meristem of lateral shoot primordium in stage of elongation and differentiation of the sprout. At the leaf base a group of meristematic cells is visible with organization corresponding to apical meristem. Below is a trace of vascular tissue (p) which will join the new shoot with the vascular tissue system of the main shoot. $\times 750$

Plate 4

Tissue differentiation in light sprout apex from potato of variety Uran. A. Apical meristem of shoot. $\times 750$. B. longitudinal section through shoot apex at stage of sprout elongation and differentiation of sprout, organization of apical meristem is visible (phot. A), procambium and differentiating strands of vascular tissue (phot. C and D). $\times 300$. C. Strands of differentiating inner phloem at level of 3rd leaf primordium. $\times 750$. D. Protoxylem and protophloem from lower part of sprout. $\times 750$

Plate 5

Anatomical structure of potato light sprout on cross sections

Phot. A, B and C show fragments of sprout tissue in stage of elongation and differentiation, phot. D presents the cross section of the sprout shoot in stage of dominance of basal part. A. Cross section through apical part of sprout. Continuous ring of vascular tissue. Differentiating vascular tissue bulges at some places into the pith area. In these groups of cells metaxylem vessels are visible. $\times 300$. B. Cross section through apical part of sprout, showing later steps of differentiation to rings of vascular tissue. Bicolateral bundles, smaller and larger alternating ones are visible. The segment between the bundles are linked with inter-bundle cambium. $\times 50$. C. Cross section through basal part of sprout. Noteworthy are the extensive groups of inner phloem (fw) forming a ring the width of which (marked by arrows) frequently is larger than that of the remaining vascular tissue. Cambial zone built of 3-4 cell layers. Outside the cambium lie groups of outer phloem and single phloem fibres. Inside the shoot vessels and other xylem elements (marked with thicker arrow) are deposited radially. $\times 300$. D. Cross section through apical part of shoot. Cambial zone (ka) consists of 2-3 cell layers. On cross sections dispersed inner phloem groups (arrow) can be

seen at a large distance from the cambium, and the outer phloem groups bordering on the cambium (phloem fibres denoted by "w"). $\times 1200$

Plate 6

Adventitious roots primordia and stolon in basal part of light sprout. A. Longitudinal section through light sprout of variety 'Krokus' in stage of dominance of basal part of sprout. The apical part of the sprout (a) is surrounded by leaf primordia. In the basal part (b) a group of adventitious roots (k) is visible. $\times 12$. B. longitudinal section through light sprout. At base of scaly leaf a lateral shoot primordium — stolon is visible (marked by arrow). $\times 50$

Plate 7

Ultrastructure of apical meristem cells in light sprouts of healthy potato plants. a. Fragment of meristematic cell from apical region of sprout, variety Uran. Centrally situated cell nucleus (n) with well demarcated nuclear envelope; heterochromatin distributed peripherally. Numerous mitochondria (m), ribosomes, proplastids (p) small vacuoles and fragments of granular ER are visible. In the thin cell walls plasmodesms are present. $\times 12\,000$. b. Prolamellar body (p), plastoglobules and starch grains (s) in differentiating plastid of parenchymal cell from apical meristem region. $\times 40\,000$

Plate 2

B5

B4

B1

B3

B2

Plate 4

Plate 6

Plate 8

Ultrastructure of phloem element in light sprouts of healthy potato plants
 a. Fragments of differentiating protophloem elements. Sieve elements (se) exhibit nonuniformly thickened wall and greatly extended dictyosome system. $\times 12\,000$.
 b. Fragment of differentiating sieve element. In cell protoplasm agglomeration of phloem protein tubules is seen (asterisk) and dictyosomes with vesicles. Along irregularly thickened cell wall microtubules (mt) are located. $\times 48\,000$

Plate 9

Ultrastructure of phloem elements in light sprouts of healthy potato plants. a. Differentiated sieve plate. Pores lined with callose (ca) and filled with phloem protein filaments—p-protein (p-p). $\times 24\,000$. b. Cross section through differentiated sieve elements. Phloem protein filaments visible in them, spherical plastids and paramural SER. In neighbouring parenchymal cells large cell nuclei (n), plastids, mitochondria and ER are seen (er), the latter forming parallel layers of cisternae.

$\times 12\,000$

Plate 10

Diagrams of light sprouts from potato plants infected with PLRV prepared on the basis of morphological and anatomical examination of the successive developmental stages. The sprouts were obtained after artificial pregermination from January to the end of March. A. Developmental stage of healthy sprout. B. Sprouts of infected plant. Type I — no sprouting of eyes in tuber (variety 'Apta'). Type II — filamentous sprout formation (varieties 'Apta' and 'Bolko'). Type III — light sprout formation in the development of which similar developmental stages can be distinguished as in healthy plant sprouts, their size is, however, reduced

Plate 11

Anatomical structure of light sprouts of potato plants infected with PLRV, the development of which occurs according to type III (diagrammatically shown in Plate 8). A. Cross section through apical part of sprout of variety 'Krokus' at stage of dominance of apical part. Vascular tissue distribution is visible. In vascular bundles greatly extended zone of inner phloem which occupies a three times larger surface than the remaining vascular tissues. $\times 50$. B. Inner phloem necroses (n) in basal part of sprout. $\times 1200$. C. Irregular distribution of inner phloem groups in greatly extending bicollateral bundle. Necrosis of sieve tubes and companion cells is visible and hypertrophy of parenchymal cells. $\times 120$

Plate 12

Ultrastructure of apical meristem cells in potato plants infected with PLRV. a. Fragments of meristem cells from apical region of light sprouts, variety Apta. In one of the cells a late telophase is seen; daughter nuclei surrounded by nuclear envelope. In centre of mother cell, cell plate is still incomplete (denoted by arrow), close to it are microtubules. Neighbouring cells in interphase. $\times 15\ 000$. b. Cells of central meristem region. One of cells in stage of metaphase; in cell centre fragment of metaphase plate is visible (marked by arrow). In neighbouring cells within the cytoplasm there are numerous vacuoles, proplastids in which usually relatively large single starch grains may be found, mitochondria and fragments of ER. $\times 7500$

Plate 13

Ultrastructure of phloem cells in light sprouts of PLRV-infected potato plants. a. Picture of necrosis of phloem parenchyma cell. Asterisk denotes strongly extended cell wall. In protoplast deposits of osmophilic substance. $\times 40\ 000$. b. PLRV (v) particles, aggregations of rolled up membranes, vesicular structures and minute filaments in electron clear part of cytoplasm of companion cell. $\times 48\ 000$. c. PLRV particles in necrotized sieve element. Arrows show fragments of protein-lipid membranes on which virus particles are deposited. $\times 120\ 000$

Plate 14

Ultrastructure of phloem cells from light sprouts of potato plants infected with PLRV. a. Fragments of sieve element and companion cell. Planting section through wall joining sieve element with companion cell shows the specific junction of the cells, since the plasmodesms on the companion cell side constitute a widely branched system. $\times 12\ 000$. b and c. Grouping of phloem protein filaments and vesicular structures visible close to sieve plate. Contents of vesicular structures are built into the cell wall. c — $\times 24\ 000$, b — $\times 60\ 000$

Plate 15

Ultrastructure of phloem cells of light sprouts from potato plants infected with PLRV. a. Tubular structures and myelin bodies in phloem parenchyma cell. Arrows indicate tubular structures inside ER channels. $\times 60\ 000$. b. Tubular structures (indicated by arrows) close to sieve plate. $\times 48\ 000$

from the apical meristem and from strands of vascular tissue. Tissue fragments were fixed in Karnovsky solution (1965) consisting of a mixture of 3 per cent glutaraldehyde and 4 per cent paraformaldehyde in cocodylate buffer pH 7.2 for 2 h at room temperature. The material was postfixed in 1 per cent OsO_4 for 2 h at 4°C , dehydrated in alcohol, acetone and propylene gradients and embedded in Epon 812. An LKB ultramicrotome was used for cutting ultrathin sections which were additionally stained with lead citrate and uranyl acetate according to Venable and Coggeshall (1965). Observations and documentation were recorded with a JEM 100 C electron microscope.

RESULTS

After the end of the vegetation season the tuber consists mainly of ground tissues, the cells of which are mostly differentiated and filled with storage material. The tubers include also primary meristematic tissues localised in what is called eyes. These tissues after the end of quiescence become active. If the process of eye sprouting occurs under light at appropriate temperature and humidity, characteristic light sprouts are formed (Plate 1).

The following stages were distinguished in the development of light sprouts on the basis of the morphological and anatomical observations performed (Plates 1, 2):

(1) quiescent eye stage — inactive apical meristem covered with scaly leaves,

(2) stage of sprout formation — the apical meristem becomes active, it produces numerous leaf primordia and the development of the vascular system from procambium strands starts,

(3) the stage of elongation and differentiation of the sprout — elongation of the light sprout is accompanied by its differentiation to the apical part covered with numerous leaf primordia and the basal part on which root and shoot primordia form above the scars left by the scaly leaves,

(4) stage of dominance of the basal part — intensive growth and differentiation of this part of the sprout, development of adventitious roots and stolons,

(5) stage of dominance of apical part — intensive growth of internodes and development of leaves proper. The sprout differentiates to an above ground shoot growing intensively and an underground part which does not elongate at this stage.

It results from anatomical observations that the developmental stages of the light sprout are a manifestation of the activity of the apical meristem with differentiation of the daughter cells, and particularly the development of the vascular system.

The resting eye should be considered as apical meristem of the shoot since the tuber is formed by the apical part of a stolon. The apical meristem of the resting eye is directly connected with the vascular system of the tuber (Plate 2B₁).

When the apical meristem starts its activity (stage 2), a bulging of the leaf primordia is observed (Plate 2B₂ and Plate 4A). Their initiation occurs in the zone at the boundary of the apical part (to the youngest leaf primordia). The apical part is the region of meristematic cells among which initials are present.

The lower part of the subapical region (the part below the youngest leaf primordia) conditions the shoot length. At this stage of development of the light sprout a certain elongation takes place (Plate 4B), mainly as the result of formation of new leaf primordia, while the internode segments remain very short. The peripheral meristem distinguishable in the subapical part gives rise to the primary vascular system and the cortical part of the shoot. In the lower subapical part of the apical meristem differentiation starts to procambium and primary cortex of the shoot. The central meristem gives rise to the pith parenchyma (Plate 3A).

Procambium gradually differentiates from the basic meristem, but it is difficult to establish the level of the first differentiation. Procambium initiation is an important moment in the differentiation of the shoot as a whole. The procambium cells remain as eumeristematic tissue, whereas the future primary cortex cells undergo vacuolisation (Plate 4A, B).

On the cross section the differentiating vascular zone is of the shape of a ring with small intrusions into the pith. The procambium cylinder increases its dimensions by cell divisions and by enlargement of the cells. The increase in size of certain elements is most noticeable at the boundary with the pith (Plate 5A). In this way small groups of cells are formed which become inner phloem initials. The cells in the centre of the procambial cylinder are of very regular shape. The first appear discontinuously at the site of procambium bulges and they constitute cambium initials. The amount of xylem — protoxylem elements which exhibit secondary bulges in the form of rings or of a loose spiral (Plate 4D) increases. In this way there arise from procambium in the early period of differentiation: protoxylem, cambium and protophloem. The phloem groups appearing in inner- and outermost regions of the procambium, hence the bundles formed are bicolateral. The phloem elements are difficult to distinguish up to the moment when the sieve tubes differentiate. The differentiation process occurs first in the inner phloem (Plate 4C).

On cross sections irregular differentiation is observed in the pro-

cambial cylinder. Six groups of vascular tissue arise, three of which are larger than the others. Their development and specialization occur much faster (Plate 5B). Differentiation spreads to the inter-bundle regions by way of appearance of cambium and primary groups of phloem initials. The inter-bundle cambium does not form simultaneously on the whole periphery, but at various sites, it then merges and fills the gaps between the large groups of vascular bundles. The phloem groups are visible outside and inside the cambium. The outer groups are smaller and more compactly arranged, while the inner phloem groups are usually more distant from the cambium and irregularly distributed. Differentiation of the vascular tissue central cylinder occurs further as follows. Metaxylem elements arise. Part of the cells form radial rows. Outer and inner phloem groups increase in size since the number of cellular elements in them increases. Phloem fibres appear latest. At first they occur singly, later their number increases and they frequently form cell groups. The cell walls are greatly thickened, but do not undergo lignification (Plate 5D). Part of the central cylinder cells preserves the character of parenchymatous unspecialised tissue. The intervascular cambium is 2-3 cell rows wide.

In the third developmental stage of the light sprout a distinct apical and a basal part can be distinguished (Plate 6A). The development of the apical part is determined by the presence of apical meristem. The gradually pushed back distal part of the shoot forms according to a different pattern. Characteristic is the enlargement of this part resulting from the function of the cambium (secondary increase in size occurs), and from the increase of the pith (Plate 5C).

At the base of the reduced scaly leaves exogenous lateral shoots form that is stolon (Plate 6B). These are initiated relatively late and they are not at once linked with the main shoot vascular system. These are called disconnected meristems (Plate 3B). The tissue below the lateral shoots undergoes vacuolisation. Procambium differentiates basipetally. The organization of the stolon apex is similar to that of the shoot apical meristem, but it has a markedly lower number of leaf primordia. These primordia do not in the future develop to leaves proper.

In this part of the shoot initiation of adventitious roots occurs (Plate 6A). The roots form endogenously. It would seem that the processes of initiation of stolon and adventitious roots in the basal part of the sprout have the following course:

- (1) in the axil of the scaly leaves of the basal part of the shoot the apical meristems of stolons develops;

- (2) the basal part increases its volume owing to cambium activity, (the stolon procambium unites with the bands of vascular tissue of the sprout);

(3) outside the forming vascular tissue band, cambium produces groups of meristematic cells which under suitable conditions begin to divide intensively to form adventitious roots primordia;

(4) in the fourth development stage of the light sprout the development of the basal part dominates. This part extends greatly, owing to the activity of cambium and the pericore zone. The earlier initiated adventitious roots and lateral shoots develop. The apical part, on the other hand, elongates only slightly and the oldest leaf primordia begin to develop into leaves proper (Plate 2B₄).

In the fifth stage the apical part grows intensively, owing to elongation of the internode segments. The leaves proper develop rapidly and new leaf primordia are formed by the shoot apical meristem. The apical part of the sprout takes on the typical appearance of an above ground potato shoot. In the basal part no major changes occur as compared with the state described in stage four. This part fulfills the function of an underground potato organ (Plate 2B₅).

Ultrastructure of apical meristem cells

Apical meristem cells were observed in various developmental stages of light sprouts. Their ultrastructure indicates a high metabolic activity. Cells of this region, both in the apical and subapical part contain very numerous free ribosomes in the cytoplasm and dictyosomes around which vesicular structures are agglomerated. Mitochondria are numerous. Their cross sections are usually rounded or oval.

Constricted mitochondria may also be seen (Plate 7A). In the apical part of the apical meristem endoplasmic reticulum (ER) membranes are scarce, whereas in the subapical part its amount increases.

The cytoplasm contains minute vacuoles usually filled with membranous or fibrous structures. Merging of the tiny vacuoles is noted. Within the cytoplasm there are no encapsulated lipid drops.

In most protoplastids minute starch grains are visible. In cells derived from the apical meristem mother cell proplastids undergo differentiation. There appear in them plastoglobules and prolamellar bodies from which the inner membrane system forms (Plate 7B). Interphase nuclei occupy a large part of the cell. They have a strongly staining fine-grained structure. Heterochromatin usually lies immediately under the nuclear envelope. In peripheral meristem cells there usually are two nucleoli and in the remaining apical meristem cells a single centrally situated one.

The walls of most cells are thin and contain numerous plasmodesms. The thickness of their walls is not equal on the whole cell periphery.

Frequently close to the cell wall paramural bodies are visible indicating an extension of the walls. There are no intercellular spaces.

Ultrastructure of phloem cells

Procambium which develops from peripheral meristem gives rise to vascular tissues. The procambium cells preserve their eumeristematic character. As the result of their division part of the daughter cells differentiate — to vascular tissue conducting assimilates, called phloem. From one mother cell of a sieve tube member there arises by longitudinal division a sieve element and a companion cell. The process of sieve element formation is associated with a number of successive transformations of this cell:

(1) characteristic thickening of cell walls (Plate 8a, b), within the cytoplasm close to the wall numerous dictyosomes may be seen and microtubules are located along the walls,

(2) the cell nucleus disintegrates and agglomerations of tubules of various sizes with phloem protein aggregate in the cytoplasm (Plate 8b),

(3) at the site where in the future pores of the sieve plate will form, that is around the plasmodesms, the amount of callose increases in the cell walls,

(4) tonoplast and ribosomes disappear, the remaining organelles including endoplasmic reticulum translocate close to the walls; the ER forms characteristic stacks,

(5) the differentiated sieve element with the sieve plate, the pores which are lined with callose, has not numerous ER cisternae situated close to the walls, mitochondria and plastids in which the number of membranes of the internal structure decreases. Within the lumen of the sieve element there are phloem protein filaments, the phloem protein tubules disappear (Plate 9a, b).

The process of differentiation of the sieve element is associated with the disappearance of certain structures and the formation of others. ER occurs the whole time while the cell is functioning, but its amount, structure and distribution change with the successive steps of sieve element differentiation. At first the amount of granular ER cisternae is small. They are located at various sites of the cytoplasm. Then the ER amount increases. When the sieve element passes to the state of functional maturity, the ER cisternae become greatly flattened and occupy positions close to the walls next to the plasmalemma. They form parallel or perpendicular stacks. Ribosomes completely disappear on the ER membranes. In mature sieve elements only a small amount of tubular endoplasmic reticulum is present peripherally disposed. Mitochondria and plastids also are present in the course of functioning of the sieve element. Mitochondria do not undergo noticeable structural

changes, but plastids vary from elongated oval forms with a developed lamellar system and few starch grains to characteristic plastids of sieve elements. These plastids are round on cross-sections and they have a reduced inner membrane system. The few membranes present are circularly arranged just under the plastid inner membrane. Starch grains are absent.

In the companion cell only the cell wall joining it with the sieve element undergoes modification. The wall expands unequally. It contains numerous plasmodesms joining it to the sieve element. In the cytoplasm of the companion cell numerous ribosomes are seen. In early stages of sprout development endoplasmic reticulum was observed in parallel and concentric arrangement in the companion cells and phloem parenchyma (Plate 9b).

Morphology and structure of light potato sprouts in PLRV-infected plants

The following types of sprouting of the tuber eyes was observed (Plate 10):

- (1) no sprouting of eyes,
- (2) formation of filamentous sprouts,
- (3) formation of light sprouts in the development of which stages analogous to those recorded in healthy plants could be distinguished.

Observations conducted from January to May on plants infected with PLRV virus exhibiting the first type of sprouting indicate that the eyes of tubers remained in the resting stage. The structural organisation of eyes from tubers which did not sprout showed no differences as regards the apical meristem in resting stage in healthy tubers. At early dates of observation no necroses were found within the apical meristem.

The stages of development of the filamentous sprouts are illustrated in Plate 10 — type II. As compared with control material, no differentiation or developmental differences could be seen in the parts described as apical and basal. In the successive stages the filamentous sprouts showed rapid elongation of the internodes and inhibition of leaf development. The latter take the shape of scaly leaves and their basal part does not develop. A very long thin shoot forms which does not produce adventitious roots or stolons. The apical meristem shows no significant structural differences as compared with the control material. The number of leaf primordia is, however, smaller and elongation growth of the internodes occurs, demarcated by the forming leaf primordia. Necroses are observed involving at first only vascular tissues. The necrotic region expands sometimes to the apical meristem.

In the third developmental type of the light sprouts from plants

infected with PLRV, 5 stages of development can be distinguished similarly as in the control material.

The morphological differences concern inhibition of sprout growth and in part of the plants of this group a less pronounced difference in the development of the basal part.

Anatomical observation indicate that in the development of the vascular system the following changes occur:

(1) the distribution of vascular tissues characteristic for the apical part of the sprout, that is the above ground shoot, is disturbed (Plate 11A, C).

(2) necroses appear involving groups of phloem cells, mainly of its inner part (Plate 11B).

(3) in the regions of necrotic tissue hypertrophies appear.

Observation of the ultrastructure of cells of the apical meristems of light sprouts from potatoes infected with PLRV did not reveal the presence of virus particles in any of the distinguished developmental types. In type I cell divisions were not noted and the cell structure showed stronger vacuolization (Plate 12b). In types II and III various stages of mitotic division were observed, formation of fragmoplast and of the cell wall (Plate 12a) similarly as in uninfected sprout meristems.

Differentiation of vascular tissue in light sprouts infected with PLRV

The development of the structural phloem elements in light sprouts of PLRV-infected plants is determined by the presence of the pathogenic agent. Spherical virions observed in sieve elements 27-30 nm in diameter form irregular aggregations. In the companion cells particles in the form of inclusions are visible with a regular arrangement and particle size about 25 nm. The dimensions of the particles in necrosed cells were 27-30 nm in diameter (Plate 13c) and in the regular inclusions 25 nm (Plate 13b). Necrotic processes affecting phloem cells are noted several times more frequently than in the control material (Plate 13a).

In the differentiating and differentiated sieve elements sporadically PLRV particles were seen.

Phloem protein tubules and numerous vesicular structures of dictyosomal origin appear in early stages of differentiation of the sieve element.

No changes were observed in the cell nucleus.

When the sieve element passes to the stage of functional maturity, there appear phloem protein filaments called p-proteins. Beside them there are numerous vesicular structures, myelin bodies and paramural agranular ER as well as mitochondria and plastids (Plate 14a, c).

Close to the sieve plate there usually is a large number of phloem protein filaments (Plate 14c).

In the sieve element the thickness of the sieve plates increases by superposition of additional callose layers or other materials which originate from larger vesicular structures (Plate 14b). The contents of these structures are incorporated into the cell wall. Their membrane merges with the plasmalemma.

The sieve elements, companion cells and phloem parenchyma cells undergo necrosis. In this process the cell organelles degenerate. The protoplast content is highly osmophilic. On the cell walls thick callose layers are deposited as well as other unidentified wall materials in which the rolled up protein-lipid membranes are embedded. The volume of the cell lumen decreases considerably. The pathogenic process evoked by the PLRV particles is the cause of formation in the potato phloem cells of structures which do not occur in the ontogenesis of cells of healthy plants. To these belong tubular structures resembling in size microtubules (mean ca 30 nm). This type of cytopathological structure was observed even in differentiated sieve elements beside phloem protein filaments (Plate 15b). In the investigated material of plants infected with PLRV the presence was found of tubular structures in the spaces of granular ER which formed parallel configurations (Plate 15a).

DISCUSSION

The development of every living organism is conditioned by many factors. Infection with virus causes a pathological process which may disturb the course of morphogenesis or modify it.

The literature gives no data on the development of light sprouts from healthy tubers and those infected with PLRV. In the present study the successive development stages have been established on the basis of morphological and microscopic observations. It results from these investigations that structural and functional differentiation of shoots to above ground and underground ones occurs already in the early stages of their formation. The development of these two parts follows a different pattern: the development of the apical part is determined by the presence of apical meristem and procambium; the basal part develops owing to the activity of cambium. Development of procambium from the ring of meristematic tissue and further the formation of a system of vascular tissues has a similar course as in all angiosperms (Esaü, 1969) and occurs in agreement with the description of Artschwager (1918a) of the young potato shoot.

The differences in the mode of sprouting of eyes from PLRV-in-

fectured tubers, and particularly the absence of sprouting indicated the possibility that the virus particles are located in the apical meristem cells.

The problem of the presence of viral particles and inclusions produced by them in the apical meristem cells is subject to controversy in the literature. It was long believed that meristems are not damaged by viruses or only slightly (Esau, 1938). Later investigations, however, supplied evidence of the presence of viral particles or inclusions in meristematic cells (Smith and McWhorter, 1957; Smith and Shlegel, 1964; Paccini and Cresti, 1977; Carroll, 1974; Walkey et al., 1968).

The results of studies, however, indicate more frequently that the virus does not infect the meristems, and plants free of virus are obtained by the method of tissue culture (Kassanis, 1957; Solberg and Bald, 1963; Schmidt and Grahl, 1976).

Observations of the ultrastructure of apical meristem cells from light sprouts infected with PLRV did not demonstrate virus particles or cytopathological structures. The reason why meristems are usually free of virus is unknown. A number of explanations has been suggested (Matthews, 1970; Loebeinstein, 1972), but it is probable that the specific but little known metabolism of meristematic cells may be the reason why viruses are seldom detected in these cells.

The anatomical symptom of the leafroll diseases, described for a long time in the literature (Quanier, 1913; Artschwager, 1918 b) is necrosis of the phloem cells. Necrotisation is a process which also occurs in normal development of healthy plants, however, infection with PLRV causes a drastic increase in the number of necroses in the phloem. Electron-microscopic examination of necrosed cells revealed that they are the site of occurrence of irregular virus particle aggregations (Golnowski, 1976; Garbaczewska, 1978) and allowed the follow-up of the stages of necrosis development. Necrotic changes include a thickening of the cell walls (Kim and Fulton, 1973; MacMullen et al., 1977). The chemism of the material built into the thickened walls has not been established. The reaction for lignin does not always give positive results (autoradiographic investigations, Bassi et al., 1974). Frequently a positive reaction for lipids was noted. In these walls callose deposits are also observed (Esau, 1968; Tu and Hiruki, 1971; Spencer and Kimnis, 1971, Allison and Shalla, 1974). The chemism of the cell walls seems to be dependent on the kind of cells undergoing necrosis.

In the protoplast of necrotising cells degeneration processes of the cell structures occur. Cells at the border of necroses, on the other hand, have unchanged protoplast. Investigations on other viruses showed that

in such "border" cells there is no infected RNA, moreover, the cells are resistant to renewed infection (Loebenstein et al., 1977). The process of necrose formation could in this way be a defence mechanism of the plant since it not only restricts the spread of virus particles (virus does not translocate in dead cells), but arouses immune reactions in the plant.

The problem of the origin and role of the tubular structures in phloem cells of PLRV-infected plants is also controversial. It is difficult to explain what type of proteins is their component. It is supposed that these proteins may be the crystalline form of:

- (1) degenerated cytoplasm components,
- (2) excess protein of virus capsid, which without the participation of nucleic acid may produce tubular forms,
- (3) newly synthesised proteins — the result of gene expression without its regulation — which may be enzymatic protein or some other protein produced in excess.

Literature brings information that the tubular structures have been observed after viral infection (Kim and Fulton, 1975; Martelli and Russo, 1977; Endelman, 1976), and although their role and origin are unknown, the presence of these structures may be considered as a cytological signal of viral infection. The presence of these structures in ER indicates that in the cells of infected plants ER may take part in the transport and aggregation of protein subunits into tubular forms.

Streszczenie

HISTOGENEZA KIEŁKÓW ŚWIETLNYCH ZIEMNIAKA ROŚLIN ZDROWYCH I PORĄŻONYCH WIRUSEM LIŚCIOZWOJU — PLRV

Opisano rozwój kielków świetlnych ziemniaka roślin zdrowych i porażonych wirusem liściozwoju.

Kielki świetlne tworzące się z oczek bulw zdrowych różnicują się we wczesnych stadiach rozwoju na część apikalną i bazalną, charakteryzujące się odrębną dynamiką wzrostu. Rozwój części apikalnej uwarunkowany jest funkcjonowaniem merystemu wierzchołkowego pędu i prokambium. Daje to początek ulistnionemu pędowi nadziemnemu. Rozwój części bazalnej uzależniony jest od funkcjonowania kambium. Z tej części powstaje pęd podziemny. Obserwowano tu procesy inicjacji korzeni przybyszowych i stolonów.

W rozwoju kielków świetlnych porażonych PLRV opisano szereg anomalii. Obserwowano gwałtowny wzrost liczby nekroz we floemie. W komórkach merystemu wierzchołkowego roślin porażonych PLRV nie znaleziono cząstek wirusa. Kuliste wiriony o średnicy 25 nm zidentyfikowano we floemie roślin porażonych. W znekrotyzowanych komórkach floemu cząstki wirusa tworzyły nieregularne inkluzje. W komórkach towarzyszących inkluzje miały regularny układ i występowały w wyodrębnionych obszarach cytoplazmy. Inkluzjom towarzyszyły błony

białkowo-lipidowe. W komórkach towarzyszących, komórkach miękiszowych floemu a nawet w zróżnicowanych elementach sitowych występowały struktury tubularne, przypominające wielkością mikrotubule oraz proliferacje błon białkowo-lipidowych i struktury pęcherzykowate z włóknistą zawartością.

REFERENCES

- Allison A. V., Shalla T. A., 1974. The ultrastructure of local lesions induced by potato virus X: a sequence of cytological events in infection. *Phytopathology* 64: 784-793.
- Artschwager E. F., 1918a. Anatomy of the potato plant with special reference to the ontogeny of the vascular system. *J. Agr. Res.* 14: 221-252.
- Artschwager E. F., 1918b. Histological studies on potato leaf roll. *J. Agr. Res.* 15: 559-570.
- Artschwager E. F., 1924. Studies on the potato tuber. *J. Agr. Res.* 27: 809-835.
- Baerecke M. L., 1968. Zuchtung auf Virusresistenz bei Kartoffell in Mitteleuropa. Bericht über die Arbeitsgemeinschaft der Saatzuchtleiter in Gumpenstein vom 3-5 Dez. 1968, Gumpenstein, 165-181.
- Bassi M., Favali M. A., Conti G. G., 1974. Cell wall protrusions induced by cauliflower mosaic virus in Chinese cabbage leaves: a cytochemical and autoradiographic study. *Virology* 60: 353-358.
- Carroll T. M., 1974. Barley stripe mosaic virus in sperm and vegetative cells of barley pollen. *Virology* 60: 21-28.
- Cheadle V. J., Gifford E. M., Esau K., 1953. A staining combination for phloem and continuous tissues. *Stain. Tech.* 28: 49-53.
- Endelman G. M., 1976. Surface modulation in cell recognition and cell growth. *Science* 192, 4236: 218-226.
- Esau K., 1938. Some anatomical aspects of plant virus disease problems. *Bot. Rev.* 4: 548-579.
- Esau K., 1969. The phloem. *Encyclopedia of plant anatomy*. Vol. V, 2. Gebr. Borntraeger Berlin-Stuttgart.
- Esau K., 1968. Viruses in plant hosts. Form, distribution and pathologic effects. University of Wisconsin Press, Madison.
- Garbaczewska G., 1977. Ultrastructure of potato meristem tissue under virus infection. *Ann. Med. Sect. Pol. Sci.* 18, 4: 315-316.
- Garbaczewska G., 1978. Badania cytologiczne kielków świetlnych ziemniaka porażonych wirusem liściozwoju PLRV. *Zesz. probl. Post. Nauk rol.* 214: 207-211.
- Golinowski W., 1975. Ultrastructure of potato infected with potato leaf roll virus (PLRV). XII International Botanical Congress. Leningrad, 215 (abstract).
- Golinowski W., 1976. Badania cytologiczne floemu ziemniaka *Solanum tuberosum* roślin zdrowych i porażonych wirusem liściozwoju (Potato leaf roll virus). *Zesz. probl. SGGW—AR* 65: 1-60.
- Hepp R. F., 1976. Studies on potato leaf roll virus. Rozprawa doktorska, Madison.
- Karnovsky M. J., 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell. Biol.* 25: 137A.
- Kassanis B., 1957. The use of tissue cultures to produce virus-free clones from infected potato varieties. *Ann. Appl. Biol.* 45: 422-427.

- Kim K. S., Fulton J. P., 1973. Plant virus-induced cell wall over growth and associated membrane elaboration. *J. Ultrastr. Res.* 45: 328-342.
- Kim K. S., Fulton J. P., 1975. An association of plant cell microtubules and virus particles. *Virology* 64: 560-565.
- Loebenstein G., 1972. Inhibition, interferences and acquired resistance during infection. Chpt. II. Principles and techniques in plant virology by Kado J. C., Agarawal H. O. Van Nostrand Reinhold Company, New York, Cincinnati, Toronto, London, Melbourne.
- Loebenstein G., Cohen J., Shabstai S., Conts R. H., Wood K. R., 1977. Distribution of cucumber mosaic virus in systemically infected tobacco leaves. *Virology* 81: 117-125.
- Martelli G. P., Russo M., 1977. Plant virus inclusion bodies. *Adv. Virus Res.* 21: 175-266.
- Matthews R. E. F., 1970. *Plant Virology*. Academic Press. New York, San Francisco, London.
- Mac Mullen C. R., Gardner W. S., Myers G. A., 1977. Ultrastructure of cell wall thickenings and paramural bodies induced by barley stripe mosaic. *Phytopathology* 67: 462-467.
- Paccini E., Cresti M., 1977. Viral particles in developing pollen grains of *Olea europea*. *Planta* 137: 1-4.
- Murayama D., Kojima M., 1973. Studies on potato leaf roll virus in Japan. *Rept. Tottori Mycol. Inst.* 10: 731-748.
- Peters D., 1967. The purification of potato leaf roll virus from its vector *Mysus persicae*. *Virology* 31: 46-54.
- Quanier H. M., 1913. Die Nekrose des Phloems der Kartoffelpflanze, die Ursache der Blattrollkrankheit. *Meded. Landbouwhogeschool Wageningen* 6: 41-80.
- Rochow W. F., Israel H. W., 1976. Luteovirus group. *Agricultural Research Service U. S. Department of Plant Pathology, Cornell University, Ithaca, New York*.
- Schmidt J., Grahsl A., 1976. Successful application of meristem tip culture virus elimination on potato. *Pflanzenarzt* 29, 7: 75-76.
- Smith F. R., Mc Whorter F. P., 1957. Anatomical effects of tomato ringspot virus in *Vicia faba*. *Am. J. Botany* 44: 470-477.
- Smith S. H., Shlegel D. R., 1964. The distribution of clover yellow mosaic virus in *Vicia faba* root tips. *Phytopathology* 54: 1273-1274.
- Solberg R. A., Bald J. G., 1963. Distribution of a natural and alien form of tobacco tissues exhibiting localised acquired resistance to viral infection. *Virology* 27: 409-417.
- Spencer D. F., Kimmnis W. C., 1971. Ultrastructure of tobacco mosaic virus lesions and surrounding tissue in *Phaseolus vulgaris* var. Pinto. *Can. J. Bot.* 49: 417, 421.
- Tu J. C., Hiruki C., 1971. Electron microscopy of cell wall thickening in local lesions of potato virus M infected red kidney bean. *Phytopathology* 61: 862-868.
- Venable J. H., Coggeshall R., 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell. Biol.* 25: 407.
- Walkey D. G. A., Webb H. J. W., 1968. Virus in plant apical meristems. *J. Gen. Virol.* 3: 311-313.