Cell division and cell enlargement during potato tuber formation

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Received 12 May 1997; Accepted 7 November 1997

Abstract

Cell division and cell enlargement were studied to reveal the developmental mechanism of potato tuberization using both in vivo and in vitro culture systems. Distribution of cells in S-phase was visualized by immunolabelling of incorporated bromodeoxyuridine (BrdU). Mitosis was detected in DAPI (4,6-di-amidino-2-phenylindole) or toluidine blue-stained sections. Timing and frequency of cell division were determined by daily cell counting, and cell enlargement was deduced from measurements of cell diameters.

Under in vivo condition, lateral underground buds developed into stolons due to transverse cell divisions and cell elongation in the apical region of the buds. At the onset of tuber formation, the elongation of stolons stopped and cells in pith and cortex enlarged and divided longitudinally, resulting in the swelling of the stolon tip. When tubers had a diameter of 0.8 cm, longitudinal divisions had stopped but randomly oriented division and cell enlargement occurred in the perimedullary region and continued until tubers reached their final diameter.

In vitro tubers were formed by axillary buds on single node cuttings cultured under tuber-inducing conditions. They stopped growing at a diameter of 0.8 cm. Pith and cortex were involved in tuberization such as that found during the early stage of in vivo tuberization (< 0.8 cm in diameter). The larger size of in vivo tubers is, however, due to further development of the perimedullary region, which is lacking in in vitro conditions.

Key words: Cell division, cell enlargement, DNA synthesis, in vitro culture, potato, tuber formation.

Introduction

The formation of potato tubers comprises two different aspects: (a) the morphological development of the tuber, and (b) the biochemical changes resulting in the formation and storage of starch. The latter process has been extensively studied during the past decades, but the morphological aspects have received much less attention.

At the morphological level, the process of tuber formation results from two separate steps, i.e. stolon development and tuberization at the stolon tip (Booth, 1963). It is generally assumed that the longitudinal growth of stolons stops as soon as the thickening of the stolon tip starts (Leshem and Clowes, 1972; Cutter, 1978; Peterson et al., 1985; Vreugdenhil and Struik, 1989). However, other observations showed that the continued expansion of the tuber depends on the production of new internodes from the apex (Artschwager, 1924; Goodwin, 1967). Because the literature is not equivocal in this matter, it was decided to investigate the two processes by visualizing mitosis and studying the expansion of the cells to clarify the switch from stolon elongation to radial growth.

Both cell division and cell enlargement contribute to the development of tubers (Plaisted, 1957; Booth, 1963; Reeve et al., 1973b; Cutter, 1978; Peterson et al., 1985). It is not certain whether the initial radial expansion of stolons is brought about by cell divisions or by cell...
enlargement. Some workers observed that mitotic activity occurred before an increase of cell size was detected (Reed, 1910; Artschwager, 1918, 1924; Reeve et al., 1969; Duncan and Ewing, 1984). Other observations indicated that the early radial expansion was caused by an increase in cell diameter (Booth, 1963; Cutter, 1978; Peterson and Barker, 1979; Peterson et al., 1985; Sanz et al., 1996). Hence, the timing of cell division and cell enlargement is unclear.

During the development of tubers, several types of tissues are involved in cell multiplication and cell enlargement. The early idea that the new thin-walled storage tissue was derived from cambial activity (De Vries, 1878) is no longer accepted. Most later workers ascribed tuber formation to the increase in number and size of parenchymatous cells in pith, cortex and perimedullary tissue (Artschwager, 1918, 1924; Hayward, 1938; Plaisted, 1957; Reeve et al., 1969; Leshem and Clowes, 1972; Cutter, 1978; Peterson et al., 1981). However, no consistent description of these processes of tuber formation exists. Some workers mentioned cell divisions in the pith during the initial phase of tuberization (Hayward, 1938; Bradbury, 1953; Plaisted, 1957; Reeve et al., 1969).

Meanwhile others stated that the parenchyma cells in the pith divided only occasionally, and that the frequency of such divisions was negligible (Reeve et al., 1973b). Peterson and Barker (1979) did not observe longitudinal cell divisions in in vitro tubers. It is not clear whether longitudinal cell divisions occurred in the pith or not. Although it is widely accepted that the growth of the perimedullary zone produces the largest portion of the tissue in mature tubers (Hayward, 1938; Booth, 1963; Reeve et al., 1969; Cutter, 1978; Peterson et al., 1985), as far as we know, no analysis has been made of the contribution of the various tissues to the growth of the tuber at different stages of development.

In this work, in vitro tuberization was studied using single node cuttings. The uniform growth of these tubers allowed a detailed investigation of the successive events of tuberization in time and in space. Because the final size of in vitro tubers is largely different from tubers grown on the plant in soil, this study was extended to a comparison between these two types of tubers, in order to detect the cause of the difference in growth pattern.

Materials and methods

Plant material

Four-week-old in vitro potato plants (Solanum tuberosum cv. Bintje), propagated using stem cuttings, were transplanted to soil in a growth chamber at 20 °C. They were first treated with long day illumination (16 h) for 4 weeks and then with short day illumination (8 h) for 3 weeks. Single node cuttings without leaves were cultured (Hendriks et al., 1991) on an inducing medium (Murashige and Skoog, 1962) containing 8% (w/v) sucrose and 5 μM benzylaminopurine in darkness at 20 °C.
longitudinal and cross-sections were made. They were stained with 0.01% (w/v) Calcofluor White or 1 μg ml⁻¹ DAPI (4,6-diamidino-2-phenylindole), or both, to visualize cell walls and nuclei. Other sections were stained with 1% (w/v) toluidine blue (in 1% NaB₄O₇·10H₂O) and used for cell counting.

Several data were collected from the longitudinal sections of in vitro tubers: (a) tuber diameter (D); (b) cell numbers along the tuber diameter (n_D); (c) cell numbers along the long axis of the whole developing bud (n_L). Average cell widths were calculated by dividing the tuber diameter by the cell numbers along the tuber diameter (D/n_D).

Data collected from cross-sections of in vivo tubers included cell number and thickness of the following regions: (a) cortex; (b) perimedullary zone; (c) pith. Average cell widths were deduced by dividing the thickness of a region by the cell numbers in that region.

**Results**

**Morphological observation of tuber formation**

When single-node cuttings of potato plants were cultured in the dark on a medium with 8% sucrose and without GA, buds developed into stolons with tubers. At day 0, buds were less than 1 mm long. In the first 4 d, the buds grew to about 1 cm long stolons with about eight internodes (Fig. 1B). The two basal leaves never grew. The first internode (numbered from base to apex of the developing bud) above the two basal leaves is the only well elongated one to form the stolon. Stolon elongation stopped and radial growth of the stolon tip started at day 5. Tuber swelling was first observed around the first node above the two basal leaves. The swelling part included the 2 mm long upper part of the first internode and a part of the second internode (Fig. 1C). When the basal end of the swelling tuber was marked with ink, the position of the marked spot did not change with respect to the site of attachment of the stolon during the further period of tuber growth, indicating that the tuber developed acropetally. Tubers stopped growing between day 10 and day 15. At this stage, the average length and width of the tuber was 0.8 cm. It was formed from the 2–3 mm long upper part of the first internode forming the 1/3 basal part of the tuber, the whole second internode forming the middle part of the tuber, and the third internode forming the uppermost part of the tuber. The internodes above the third one neither elongated nor swelled (Fig. 1D).

During in vivo tuber development, the length of the stolon and the numbers of internodes along the elongating stolon were much more variable. However, the swelling was always observed in the stolon tip starting in the upper part of the last well elongated internode (Fig. 2A, B). At least eight further internodes had developed in the stolon tip before tuber swelling started, much like the development in vitro. Until the size of 0.8 cm in diameter, the morphology of the in vivo tuber was the same as that
Fig. 2. Diagram of longitudinal sections through *in vivo* grown potato tubers, showing the morphology of the stolon and tuber and the thickening of the perimedullary zone (dark-shaded area). Positions of nodes are indicated schematically. The numbers indicate the nodes. Bar = 1 cm. (A) 0.2 cm stolon, showing the continuous vascular bundle. (B) 0.3 cm tuber, showing the growth of pith (light-shaded area). (C) 0.8 cm tuber, showing the onset of growth of the perimedullary region. (D) 2.0 cm tuber, showing the thickening perimedullary region.

Development of *in vitro* tubers

**Time-course of cell division and cell enlargement:** Both cell division and cell expansion are involved in stolon elongation and tuber growth. Cell numbers along the transversal axis of tubers and along the long axis of stolons were determined by cell counting. Cell enlargement contributing to the swelling of the tuber, was measured by cell width.

Buds elongated and formed stolons from day 0 to day 4 of culture. During this period, the diameter of the stolons remained constant, i.e. around 1.5 mm (Fig. 3A), and cell width in the subapical region did not change (Fig. 3B). The number of cells along the stolon diameter was similar in all regions of the stolon and longitudinal cell divisions were not observed (Fig. 3C). In contrast, cell numbers along the long axis of the buds increased 6 times from 40 to 260 between day 0 and day 5 (Fig. 3D). Besides cell division, cell elongation also contributed to stolon elongation (Sanz *et al.*, 1996).

The development of the stolon suddenly switched to tuber swelling at day 5 (Fig. 3A). The elongation of the stolon stopped as soon as swelling started (data not shown) and the cell number along the long axis of the stolon remained unchanged (Fig. 3D). Cell width in the subapical region increased from day 4 onward (Fig. 3B), which was 1 d before the increase of the cell number along the radial axis at day 5 (Fig. 3C). Tuber size increased quickly from day 5 to day 8 because of both intensive cell widening (Fig. 3B) and frequent longitudinal cell divisions starting at day 5, reaching a peak at day 6 and ceasing around day 8 (Fig. 3C). The total cell number in pith and cortex diameters increased from 47 to 140, i.e. 3 times. After day 8, the number of cells along the stolon diameter remained constant (Fig. 3C), but cells continued to enlarge gradually till about day 15 (Fig. 3B), resulting in steady tuber growth (Figs 1A, 3A).

**Localization of DNA synthesis and cell division:** The distribution of nuclear DNA synthesis was investigated by the immunocytochemical detection of incorporated BrdU. Cell division was visualized by DAPI and Calcofluor White double staining.

BrdU-labelled nuclei were found in the whole subapical region of the stolon tip from day 1 to day 3 (Fig. 1A). They were small and close to each other. Sometimes pairs of labelled daughter nuclei were observed (Fig. 4a, b). In DAPI stained sections, it was observed that many new cells were arranged in meristem-like cell columns. All divisions were transversal and added new cells to the long axis of the stolon (Sanz *et al.*, 1996).

At day 4, some stolons still had a few BrdU-labelled nuclei in the meristem-like cell columns. However, most stolons showed more labelled nuclei at the basal part of the subapical region (Fig. 1B). From morphological
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observation, it was known that this region was the upper part of the first internode where tuber swelling started.

At day 5, hardly any BrdU-labelled nuclei were detected in the meristem-like cells in the tip of the stolon (Figs 1C, 4c). They were only observed in the swelling tuber (Figs 1C, 4d). The position of new cell walls and the orientation of the chromosomes as visualised by Calcofluor White and DAPI respectively, revealed that the divisions were longitudinal and added new cells to the transversal axis of the tuber (Fig. 5b). Such divisions only occurred in enlarged cells.

From day 6 to day 7, BrdU-labelled nuclei and mitosis were only occasionally found, and after day 8, labelled nuclei were only found in the periderm region and around the vascular tissue (Figs 1D, 4e, f). DAPI staining also showed mitosis in the periderm.

The development of in vivo tubers

Growth pattern: Before tuber formation, stolons had a normal stem structure with continuous vascular bundles along the long axis (Fig. 2A). At the beginning of tuber formation, the stolon tip swelled in the subapical region. The vascular bundles remained continuous along the long axis but became arc-shaped because of the swelling of the pith (Fig. 2B). In cross-sections, a circle of the xylem tissue was clearly observed with external phloem and internal phloem in the early stage (less than 0.8 cm in diameter) (Fig. 5c). After tubers had grown to about 0.8 cm in diameter, they became larger mainly because of increasing thickness of the perimedullary region including external phloem, xylem and internal phloem (Fig. 2C, D). The width of the cortex did not change during later stages of development (diameter > 0.8 cm) (cf. Figs 2C, D, and 6C). With increasing thickness of the perimedullary zone, the vascular tissue became irregularly arranged and the xylem and phloem elements were scattered in the whole perimedullary region (Fig. 5d).

Quantification of cell division and cell enlargement in different tissues: Cortex, perimedullary region and pith are the three major tissues of a tuber. Cell numbers and cell widths were measured in these tissues in the successive stages of tuber formation. The total numbers of cortical cells and pith cells on cross-sections increased from 35 in the stolons to 92 (about 3 times) in the tubers of 0.8 cm in diameter and then remained constant (Fig. 6A). Cell numbers in the perimedullary region, however, continuously increased during tuber development, but especially

Fig. 3. Quantification of changes in tuber diameter, cell number and cell width during in vitro tuber formation in potato. (A) Tuber diameter. (B) Average cell width. (C) Cell numbers along the transversal axis of the stolon and swelling tuber. (D) Cell numbers along the long axis of the stolon. Data are means with standard deviations of five independent measurements.
after tubers had reached a diameter of 0.8 cm (Fig. 6A). In all three tissues cell width steadily increased (Fig. 6B). Cortical cells were always the smallest and enlarged slightly. The pith cells were larger than the perimedullary cells especially in the early stage when the perimedullary cells were not growing (Fig. 6B).

Cell division and cell enlargement resulted in the enlargement of tubers. Distinguishing the three regions, the thickness of the cortical region only increased slightly in the early stage; the thickness of the pith region gradually increased and the perimedullary region exhibited the most obvious growth (Fig. 6C).

**Visualization of cell divisions in the perimedullary region:** In the early stage of in vitro tuber growth (less than 0.8 cm in diameter), the developmental progress is the same as that of in vitro tuberization. Then, immediately after the longitudinal cell division in pith and cortex had stopped, groups of meristem-like cells formed in the perimedullary region (Fig. 5e). Unlike the elongated vascular cells, these cells were isodiametric as concluded from the observation of cross-sections and longitudinal sections. Each group consisted of various cell types in a concentric arrangement: small meristem-like cells generally with a few xylem or phloem elements were in the centre; around them were several layers of the enlarging parenchyma cells which lacked starch grains; the outermost layers consisted of mature parenchyma cells filled with starch grains (Fig. 5e). Randomly oriented cell divisions were observed in the meristem-like cells, in the enlarging cells and also in the mature cells with starch grains (Fig. 5f). Besides, cell divisions were also observed in the cortical cells which divided along the tangential direction to allow further expansion of the tuber (Fig. 5g). However, cells in the pith did not exhibit divisions (Fig. 5h). BrdU immunolabelling was also applied to investigate cell divisions in the perimedullary zone, but no clear labelling was obtained because of poor uptake of BrdU in the larger tubers.

**Discussion**

Potato tuber formation consists of two different morphogenetic steps: stolon elongation and tuber initiation (Booth, 1963; Vreugdenhil and Struik, 1989). Contrary to Artschwager’s (1924) and Goodwin’s (1967) opinion, it was observed that the growth of tubers depends on the expansion of internodes already present in the stolon, and not on the formation of new ones (Leshem and Clowes, 1972; Cutter, 1978; Peterson et al., 1985). In both in vitro and in vivo conditions, the first indication of tuber formation is a thickening of the last well elongated internode, which is often the eighth internode counted from the apical one as described by Cutter (1978). Three internodes (the sixth to the eighth counted from the apical one) form almost the whole in vitro tubers and more than 2/3 of the in vivo tubers. The expansion and the growth of the further internodes (the first to the fifth counted from the apical one) in the upper 1/3 of in vivo tubers are caused by the subsequent growth of the perimedullary tissue in all directions.

Both cell division and cell expansion are involved in tuber development. In the literature there is a debate on the timing of these cellular events; some authors describe that cell division precedes cell enlargement (Artschwager, 1924; Plaisted, 1957; Reeve et al., 1969, 1973a; Duncan and Ewing, 1984), whereas others advocate the opposite (Booth, 1963; Cutter, 1978). The data presented in this paper clearly show that both the timing and the location of cell division and cell expansion are different in various regions of the developing stolon and tuber. Cell divisions were first seen in the apical region of the stolon as also observed by Duncan and Ewing (1984). Cells divided transversally and then elongated. However, these processes were only involved in stolon elongation. When stolon tips started to swell, transverse cell division in the apex had stopped. Meanwhile from the basal part of the subapical region upward, cells enlarged and then the enlarging cells divided longitudinally. This finding is in agreement with that of Sanz et al. (1996) who stated that cell enlargement precedes cell division during the initiation of radial tuber growth.

It is generally agreed that the growth of the in vivo tuber occurs initially in the pith and cortex, and then predominantly in the perimedullary zone (Hayward, 1938; Booth, 1963; Reeve et al., 1969; Cutter, 1978; Peterson et al., 1985). The observations here support this view. However, it was noticed that the growth in pith and cortex and in the perimedullary zone occurred in different stages and with different patterns. In the early stage of in vivo tuber formation (less than 0.8 cm in diameter), cells in the pith and cortex first enlarged and then divided longitudinally. Longitudinal cell divisions stopped when tubers reached the size of 0.8 cm in diameter, whereas cell enlargement in the pith and cortex continued throughout the whole process of tuber growth. Although longitudinal cell divisions in the pith and cortex happened in a
experiments show that cell numbers increased about 3 times in the pith and cortex of young tubers (less than 0.8 cm in diameter). Unlike the description by Reeve et al. (1969), the initial cell divisions in the pith and cortex are always parallel to the long axis of the stolon and contribute to the swelling of the stolon tip mainly in a transverse direction. Although some cell divisions were incidentally observed in the perimedullary region in the early stage, the actual growth of perimedullary region, which included a lot of cell divisions and cell enlargement, started when the tuber reached a diameter of 0.8 cm and continued until the tuber had reached its final size (3 cm in diameter in these experiments). The planes of cell divisions in the perimedullary region are randomly oriented in both cross- and longitudinal sections, resulting in the enlargement of tubers in all directions. Thus the perimedullary region formed the major portion of the mature tuber.

The morphology and the processes of cell division and cell enlargement of in vitro tubers are similar to those observed during the early stage of in vivo tuber formation (day 10 in vitro is equal to 0.8 cm in diameter in vivo). The youngest tubers have a diameter of 0.3 cm (= day 5 in vitro). But in vitro tubers stop growing at a size of 0.8 cm in diameter. Comparing the in vitro and in vivo tuber formation, it was concluded that the much larger final size of in vivo tubers is caused by the further cell division and cell enlargement in the perimedullary region in the later stage, which is lacking in the in vitro tubers.

Differences between the growing conditions of in vivo and in vitro tubers, are the presence or absence of the intact plant and the supply of nutrients. This suggests that the mother plant might provide some products to the growing tuber in vivo, resulting in the initiation and continuation of cell divisions in the perimedullary region which is lacking in vitro. It is unlikely that the absence of cell divisions in the perimedullary region is due to the depletion of the medium, since transfer experiments of in vitro tubers to fresh medium did not lead to larger tubers (data not shown). However, it is highly possible that a factor, essential for the initiation of cell division in the perimedullary region is missing in the medium. Analysis of tubers grown on single-node cuttings, with the leaf still attached, revealed that these tubers did not show cell division in the perimedullary tissue either (Xu and Ewing, unpublished data). Therefore, it is suggested that a regulating factor is derived from either the root or the shoot very short period, the results of this study did not agree that cell divisions in the pith and cortex are negligible as mentioned by Reeve et al. (1973b) and Peterson and Barker (1979). The in vitro and in vivo data from these

![Graphs showing cell numbers, cell widths and the thickness of different regions along the transversal axis of in vivo potato tubers. (A) Cell numbers. (B) Average cell width. (C) Thickness of different regions. Data are means with standard deviations of three independent measurements](https://academic.oup.com/jxb/article-abstract/49/320/573/514928/Cell-division-and-cell-enlargement-during-potato)

**Fig. 6.** Graphs showing cell numbers, cell widths and the thickness of different regions along the transversal axis of in vivo potato tubers. (A) Cell numbers. (B) Average cell width. (C) Thickness of different regions. Data are means with standard deviations of three independent measurements.

![Micrographs showing cell division during in vitro (a, b) and in vivo (c–h) tuber formation in potato. Bar=100 μm. (a, b) DAPI and Calcofluor White stained longitudinal sections of in vitro stolon and tuber. The long arrows on the right upper corner indicate the long axis of stolon and tuber. (c–h) Toluidine blue stained cross-sections of in vivo tubers. (a) Transversal cell divisions in the subapical region at day 3. The short arrow indicates the mitosis in one cell. (b) Longitudinal cell divisions in the swelling tuber at day 5. (c) Vascular tissue in in vivo tuber of diameter=0.3 cm: ep, external phloem; x, xylem; ip, internal phloem. (d) Irregular arrangement of vascular tissue (see the short arrow) due to the growth of the perimedullary region. (e) Groups of concentrically arranged cells in the perimedullary region. (f) Random cell divisions (see the short arrows) in a group of perimedullary cells. (g) Tangential cell divisions (see the short arrows) in cortical cells. pe, periderm. (h) Absence of cell division in the pith of larger tubers (diameter >0.8 cm).](https://academic.oup.com/jxb/article-abstract/49/320/573/514928/Cell-division-and-cell-enlargement-during-potato)

**Fig. 5.** Micrographs showing cell division during in vitro (a, b) and in vivo (c–h) tuber formation in potato. Bar=100 μm. (a, b) DAPI and Calcofluor White stained longitudinal sections of in vitro stolon and tuber. The long arrows on the right upper corner indicate the long axis of stolon and tuber. (c–h) Toluidine blue stained cross-sections of in vivo tubers. (a) Transversal cell divisions in the subapical region at day 3. The short arrow indicates the mitosis in one cell. (b) Longitudinal cell divisions in the swelling tuber at day 5. (c) Vascular tissue in in vivo tuber of diameter=0.3 cm: ep, external phloem; x, xylem; ip, internal phloem. (d) Irregular arrangement of vascular tissue (see the short arrow) due to the growth of the perimedullary region. (e) Groups of concentrically arranged cells in the perimedullary region. (f) Random cell divisions (see the short arrows) in a group of perimedullary cells. (g) Tangential cell divisions (see the short arrows) in cortical cells. pe, periderm. (h) Absence of cell division in the pith of larger tubers (diameter >0.8 cm).
of the intact plant, a factor which is missing in the in vitro cultures. Further studies will be needed to test this hypothesis.

Acknowledgements

The authors are indented to Henk Kieft, Elly Koot-Gronsveld and Wilma Pons-Drexhage for technical advice and support, to the Royal Dutch Academy of Sciences (KNAW) for supporting the co-operation between China and The Netherlands, and to the Wageningen Agricultural University for financial support. This work was partly funded by the European Union’s BIOTECH programme, as part of the Project of Technical Priority.

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