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# The Ontogenetic Trends of Microtuber Formation in Potato (*Solanum tuberosum* L.)

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**Abstract:** The main aim of this investigation is the understanding of how microtubers are formed out of auxiliary buds in induced conditions. In the induced buds, meristematical cells with high cytoplasmic and nuclear stain ability expand deeper into the inner sections of the buds, comparing with non-induced buds, which the area is restricted to the apical regions. The first sign of microtuberization is the increase in size of cortical parenchyma cells in lower section and also increase in mitosis divisions in inner sections of the meristems. Most of the growth rates occur in the induced medium along the length and the width of the cells. It is also considered that the diametric growth of the tubers and the base of the leaves on the tubers begin their radial growth. The cortical parenchyma cells begin forming amidon grains during their vacuolar extension at extending the internodes much earlier than pith parenchyma cells. The extension of cells in sub-apical region plays an important role in the longitudinal growth of tubers. In the first stages, the growth of tubers results from the change in the dimensions of cortical and pith parenchyma cells, due to the reproduction of apical meristem and later, mainly from the growth of the productive tissue in the pith parenchyma. Longitudinal growth is initially grater than the growth in diameter; however with shift in the position of vacuoles and their arrangement across rather than along the tubers, the growth pattern begins to change and lateral growth catches on and exceeds longitudinal growth. In fully grown tubers, pith parenchyma cells are larger than the cells in cortical parenchyma.

**Key words:** *Solanum tuberosum* L., ontogenetic, microtuber formation, meristem cells

## INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important food resources in the world. Formation of the tubers in potato can be study from two different aspects: 1)the morphology of their growth and 2) the biochemical changes resulting in the formation and storage starch material. The latter process has been extensively studied in the past decades; however, not enough attention has been paid to the morphology of their growth (Jimenez, 2000). The ontology and structure of tubers in potato has been studies by many researchers (Desfontaines, 1998; Yu, 2000). The potato tuber has lateral leaves and buds with short internodes and radial growth of stem axial. The Stolon apical meristem is located at one end of the tuber, named Rose or bud end; the other end is called Heel or Stem. With activity beginning at the Stolon end, leaves begin growing in a helix. These leaves, along with their auxiliary buds are known as the eyes of the potato. Each eye is in fact a stem node and is comprised of an auxiliary bud covered by scale leaves.

The initials increase in the size of the sub-apical region of stolons is the result of increase in the number of cells in the pith followed by increased cell division simultaneous with cell growth. Changes in the parenchyma around the internal phloem (makkrone) and to a lesser degree, in the perimedullary parenchyma of the outer phloem, have greater effect on the growth of the tuber (Ruzin, 1999). These divisions, however, come to an end earlier than the growth in the cells. The ultimate size of the tuber is the result of the increase in the size of the cells, particularly in the innermost cells of the cortical parenchyma and around the pith. Starch is the most important convertible substance in potato tubers. Most of the starch is in the cell is simple, with eccentric helium and distinct stratification. There are sometimes compound starch granules with more than one helium. Starch granules have an enclosing membrane because they develop in certain plastids called Amiloplast. The main aim of this investigation is the understanding of how microtubers are formed out of auxiliary buds in induced conditions.

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## MATERIALS AND METHODS

In order to study the ontogenetic cellular trend of microtuber formation, the tubers, resulting from culture of the tissue were fixed in the different stages of their growth for 24 h at plant physiology laboratory of Azad Islamic University-Garmsar Branch, Garmsar, Iran in an MS induce environment with 80 g $L^{-1}$ saccharose, BAP 10 mg $L^{-1}$ (Jimenez, 2000; Kefi, 1998). Dehydration and clearing was performed with 30, 40, 50, 70, 90 and 100% (changed three times). For paraffin penetration, proportions of isopropanole: paraffin, pure paraffin and for blocking the samples, (Merck) paraplast was used. Eight micrometer longitudinal and cross-sectional layers were cut from the samples using Leica Microtome. For staining the samples, Meir hematoxciline and 70% alcoholic eosin were made use of (Xu and Vreugdenhil, 1998). After a second dehydration and transfer to xilol, the layers were stuck with Antalen adhesive. The study of tissue and cell structure of the stems, meristems and microtubers was performed with a Photo Alpha Nikon binocular light microscope and Leica image analyzer.

## RESULTS

The auxiliary buds normally have a short shoot axial attached to the stem at the lower end. at the upper end of the axis, we see the meristemic dome surrounded by two or more primordial foliars (Fig. 1A). If not in growth or induce conditions, these buds do not establish precambial and vascular relationships with lower sections and if they grow, their vascular systems do not join the vascular system of the stem. The extent of staining in the stem axial and below the meristemic dome is less than the apical region and particularly the leaf-forming flanks. Leaves begin to appear with alternative and helix leaf patterns on the sides of the meristemic dome (Fig. 1A). There are no meristemic cells in the sub-apical regions and the cells in this region begin to grow longer and exhibit cell distinction because of vacuolar extension. Basic and meristemic cells are only limited to a number of apical layers of meristem. Before any anatomical changes in the lateral meristem in the induce medium for establishment of microtubers, the first general signs are the formation of several amidon granules in the cortical and pith parenchyma cells of the shoots to which the buds are attached (Fig. 1B). Along with this differentiation it becomes possible to distinguish a limited number of the auxiliary buds from the non-induced vegetative lateral meristems because of the development of the meristemic tissue up to the sub-apical region of the buds and the extent of their stainability and their pattern of cell division. These buds grow into microtubers in later stages, they are shorter and more compact than non-induced lateral meristems (Fig. 1B) which establish procambial and vascular relationship with lower stem structures, while forming leaf premordiums with alternative arrangements.

For microtuberization, we can observe the first cells divisions in these meristems in the sub-apical regions as of the fourth day after being placed in an induce medium (Fig. 1C). Before these inner divisions, in the peripheral and lower sections of primordial foliar of induced buds, cells begin to differentiate; vacuolar systems begin to develop in them and their nuclei gradually move from the upper regions to the lower parts and closer to the inner walls (Fig. 1D). There is still no sign of amidon granule accumulation in these cells. At the same time, or a short while after, cells begin to divide in the apical section of the meristemic dome (Fig. 2A). These divisions expand in different directions, including along the longitudinal axis of the meristem, which is indicative of an increase in the number of the cells in various sections of the meristem. These divisions usually take place in the sides and marginal sections of the meristem (Fig. 2B and C). periclinial divisions are observed in the sides of the meristem and anticlinial divisions in the inner sections. Leaf-forming activities in meristems take place a short time after growth in their lower sections. All these activities take place in connection with leaf forming on the sides of the meristem. In the lower section and between the lateral and the apical regions there are sells that act as pith meristems and the resulting cells differentiate and grow quickly, forming the pith parenchyma.

Epidermal cells of the dorsal side of the premordium and the initial pattern of the leaves begin their differentiation much earlier than the ventral epidermal cells and become vacuolated; this phenomenon is also observed in the formation of mesophylle cells. During the differentiation of epidermal cells, which is simultaneous with their elongation, growing vacuoles begin to move towards the outer surface walls of these cells and cover the nucleus inside like a protective structure.

The study of several longitudinal slices shows that microtuber growth is an acropetal process beginning below the apical meristem (Fig. 3). During their vacuolar development, cortical parenchyma cells begin producing starch and amidon granules at the growing internodes below the apical region, faster and much earlier than pith parenchyma cells (Fig. 4). Considering both time and space, the delay in the formation of amidon granules in pith parenchyma cells occurs below the meristem. Likewise, accumulation of amidon granules in microtubers takes place faster on the side farther from the stem of the buds than the side closer to it. The activity of the apical

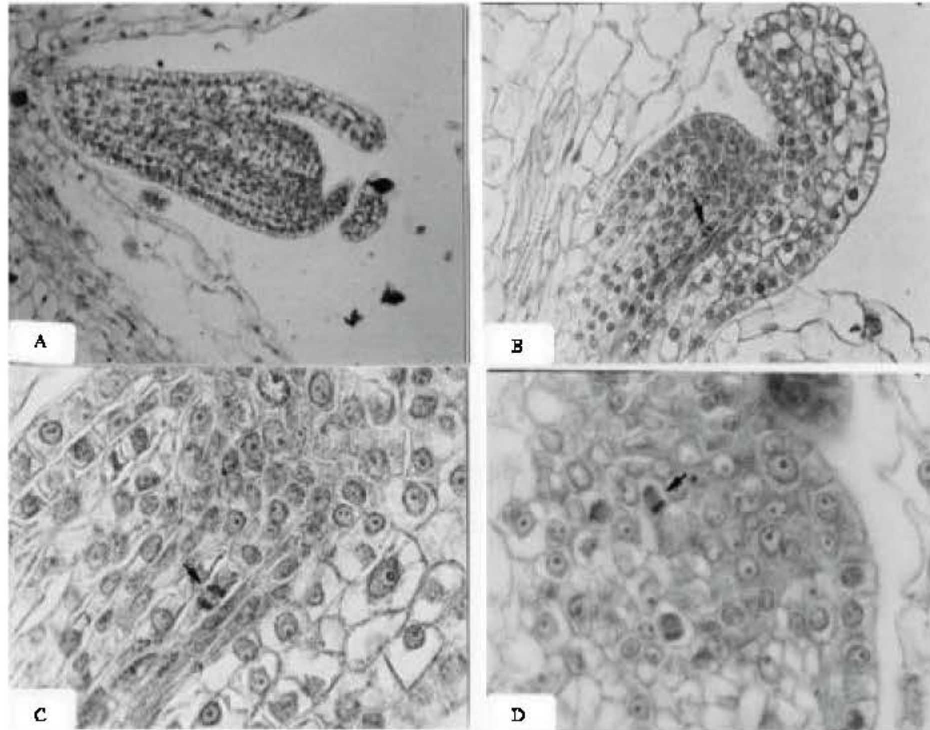


Fig. 1-A: a longitudinal section of non-induced lateral meristems with two primordial foliars of the meristemic dome. Leaf initials begin forming alternatively on the sides of the meristem(x 200). (B) Induced meristem for microtuberization. Meristemic cells have expended into eh inner sections. One primordial foliar has developed on the side of the meristem (x 200). (C) the first signs of division -periclinial or anticlinial(D) - in the innermost sections of the meristem. These two figures show cells in different stages of nucleus division (mitosis) (x 400)

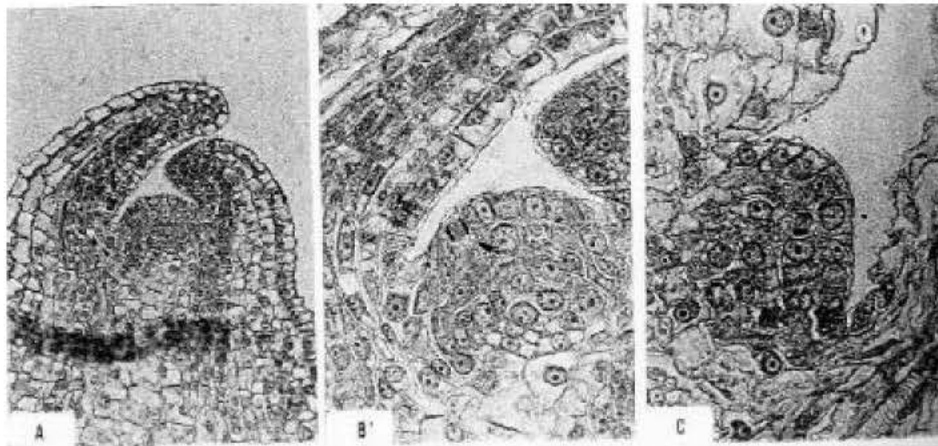


Fig. 2: Activation of the apical meristem of the microtuber and the increase in the size of the sub-apical region (A) (x 200), (B) (x 400) and (C) (x 400). In these figures it is possible to see the activity in the meristem, in the form of cell division in protodermal regions as well as areas adjacent to the apex of the meristem. It is easy to see in figure (B) that the nuclei of the protodermal and epidermal cells in making move towards the inner sections of the leaf, simultaneous with the development of vacuoles; which can be regarded as a protective system for the nucleus. In figures (A) and (C) one can clearly see the differentiation of vascular elements below the meristem and in relationship with primordial foliar

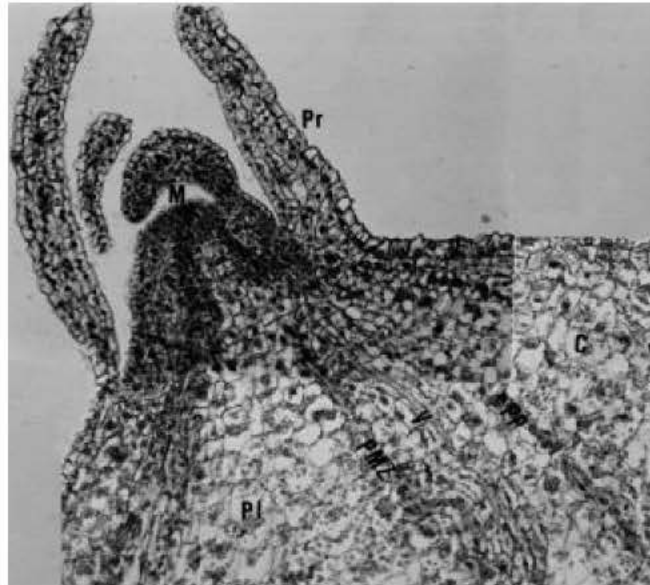


Fig. 3: Anatomical structure of the sub-apical region in growing microtubers(x200). General view of the sub-apical region-the meristemic dome (M), primordial foliar (Pr), epidermis (E), cortical parenchyma, the reproductive tissue around the external phloem (PP), Vessel (V) the reproductive tissue of the perimedullary parenchyma (PMZ), pith parenchyma (Pi). In the pith parenchyma tissue it is possible to observe the change in the growth pattern in the increase in the distance from meristem

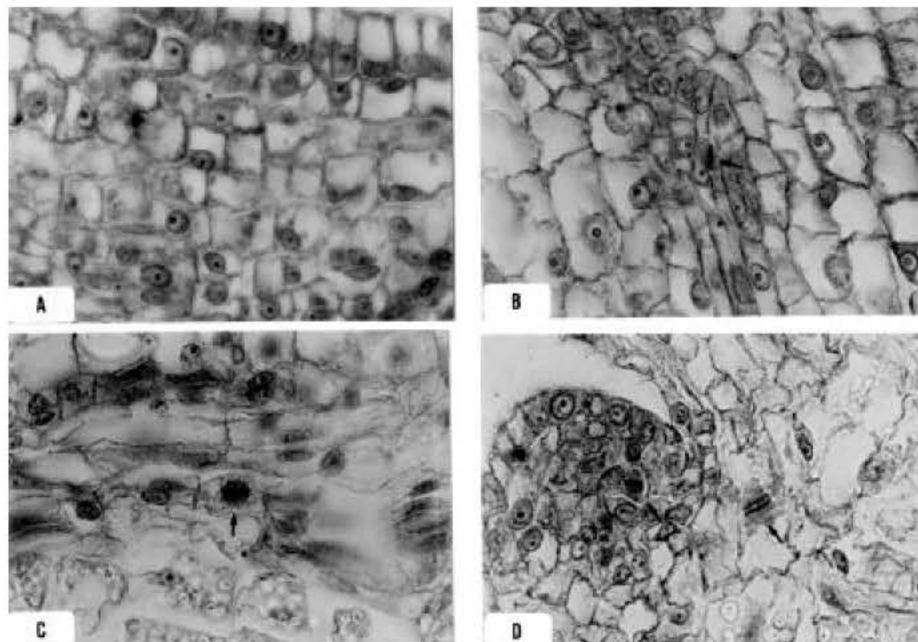


Fig. 4: (A) In the sub-apical region and in the forming cortical parenchyma, cells begin to exhibit first longitudinal and later radial growth, while producing starch. This change in the growth pattern is simultaneous with the movement of vacuoles in the cells. (B) Likewise, in the perimodullery parenchyma, cells division is one of the factors contributing to cell growth. (C) Cell division is also observed in the inner parenchyma cortical tissues. (D) Various divisions in different directions in the apical and sub-apical regions

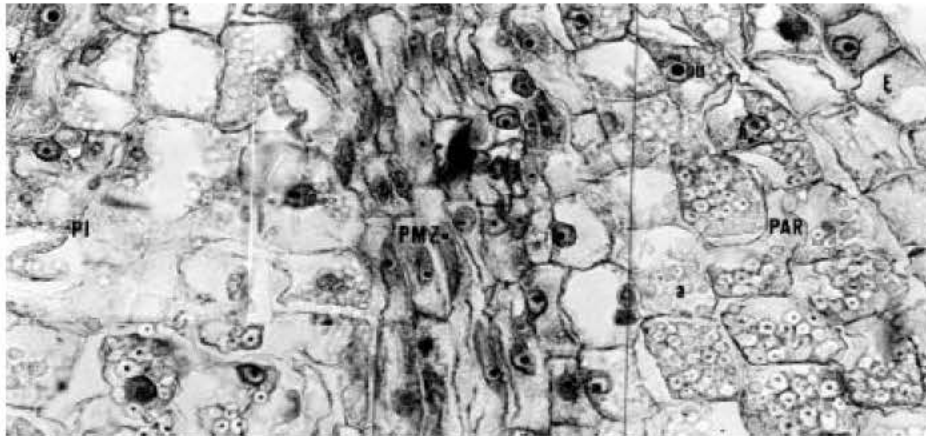


Fig. 5: The anatomic structure of inner sections of microtubers (x400). epidermis (E), cortical parenchyma (C), vascular tubes (V), perimedullary parenchyma (PMZ), pith parenchyma (Pi). There is no parenchyma around the external phloem in this area

meristem in the polar section and the growth and reproduction of parenchyma cells cause the microtubers t grow both in length and width. Since differentiation begins earlier on one side of the tuber than the other, there is no symmetry in the slices which are longitudinally cut.

The reason for the diametric growth in microtubers is the change in growth pattern in the pith and cortical parenchyma below the meristem; which initially exhibit longitudinal growth and later moving away from the apex, these cells experience more growth in width (Fig. 4A). The change in growth pattern bigins earlier in cortical parenchyma than in pith parenchyma. Under the meristem, one can observe the forming cortical and pith parenchyma as well as the vascular structures beginning to differentiate (Fig. 4B). Deeper within these tissues, simultaneous with theses changes, amidon granules begin to form, growing in number and size. It appears that besides the growth in cortical and pith parenchyma resulting from apical meristem and the change in their growth pattern along the increase in the size of vacuoles (Fig. 4B), tissues producing perimodullary parenchyma as well as the tissues producing the parenchyma around the external phloem play a part in the growth of tubers by several anti-clinal and periclinal divisions (Fig. 4C -D). The complex made up of these productive tissues and the vascular tubes between them is called perimedullary tissue.

The activity in the tissue producing the parenchyma around the external phloem stops sooner than that in the tissue producing perimodullary parenchyma, such that there is no trace of the former in the inner parts of the tubers and there is only perimodullary parenchyma (Fig. 5), the concentration of amidon granules in cortical parenchyma is greater than in pith parenchyma. Studying cross-sectional slices of forming microtubers (Fig. 5) shows that most of the amidon granules accumulate in the cortical parenchyma and pith parenchyma cells are larger with more developed vacuole systems; however, with far fewer amidon granules than those in the cortical parenchyma. The growth and division of the parenchyma cells in the cortical and pith areas causes the vascular system in the sub-apical region to change its circular arrangement (Fig. 5) and appear in broken lines with different stainability (because of the perimedullary tissues), scattered around the boundary between cortical and pith parenchyma.

Studying epidermal cells in longitudinal section shows in the distance between the growing internodes in epidermal cells, is a quick differentiation of peridermal tissue. phelogen of epidermal origin begin their activity in the form of asymmetrical periclinal divisions, which results in the formation of two rows of cells. The outer layer is made up of smaller cells, whereas the inner layer consists of larger cells. The cells in the outer layer continue their division, wile growing larger and thus make the cells in the outer layer. cells walls in this section become quickly suberinazed and die. Posterior parenchyma is formed, to a very limited extent, as a result of phelogen activity. The combination of phelum, phelogen and pheloderm have regular linear and radial arrangement. Lenticels are formed in the older parts of peridermal tissues.

In the base, young leafy premordiums (Fig. 6) cannot be observed in the apical region of lateral buds and lateral meristems are formed of subsurface divisions at a distance from the apex at the base of the initial leaf pattern and in the form of a dome.

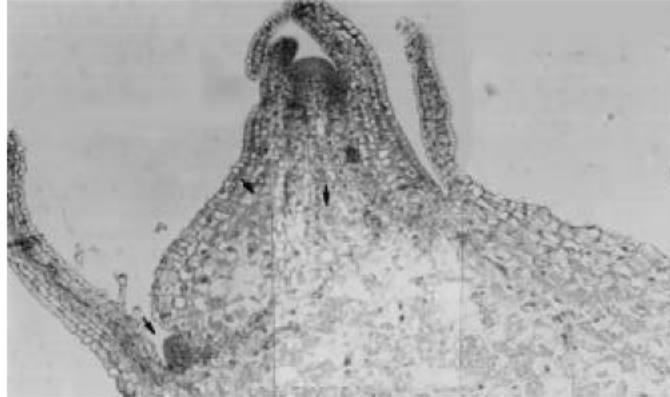

Fig. 6: A microtuber, in which it is possible to observe the apical meristem, growing internodes, and dispersion of amidon granules in cortical and pith parenchyma tissues. Along with the activity of the meristem and growth of leaves on its sides, there are no laves formed at the base of the leafy premordiums. These meristems form with a delay and dedifferentiaton at the bottom of the initial leaf patterns. The amidon cells begin the differentiations earlier in cortical parenchyma rather than the pith parenchyma (X 200)

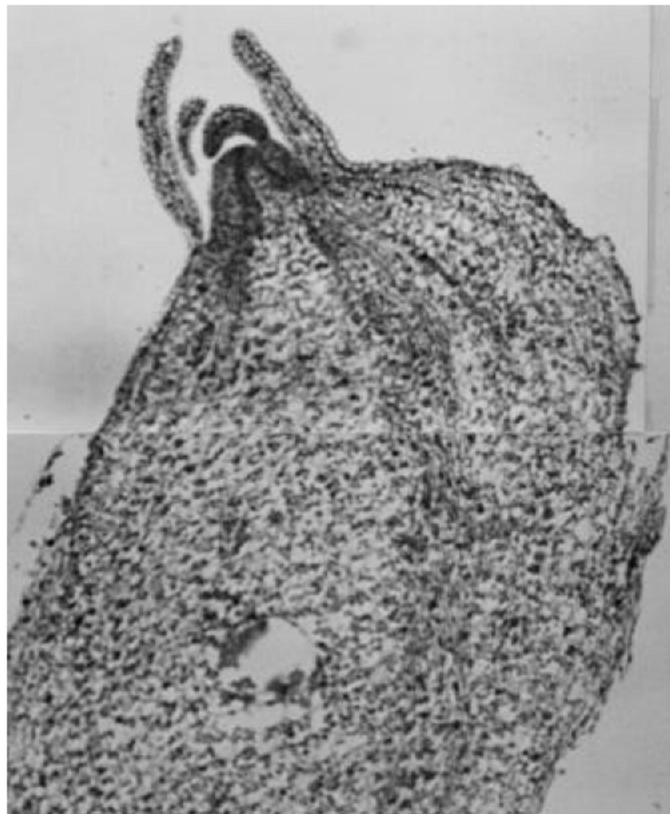

Fig. 7: The general view of the tuber showing the growth of cells, accumulation of amidon granules and pericombrium differentiation in the apical region. The figure clearly shows that the growth of the tuber is asymmetrical. The cells on the side farther from the stem grow faster below the meristem rather than the cells closer to the stem; hence the asymmetrical growth of the tuber. (x200)

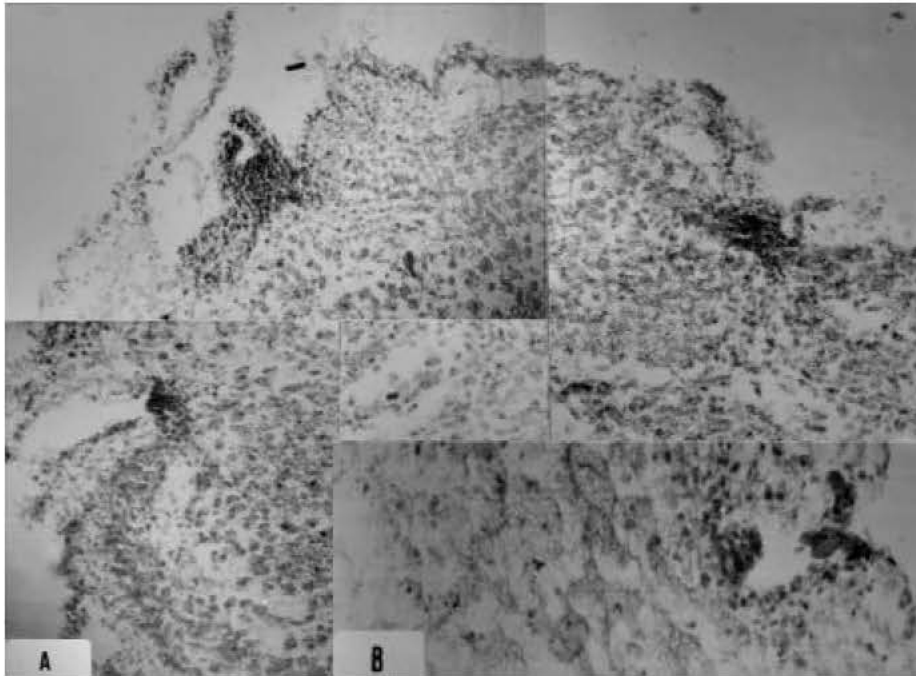


Fig. 8: (A) (x 100) and (B) (x 200) the comparative position of the apical meristem and lateral buds in fully grown microtubers, protected by scaly leaves and reserve parenchyma tissue

In the beginning stages, the increase in the number of cells is greater along the length of the tubers, than across their width (Fig. 7), but with the activation of the perimedullary section and with the increase in the number and sizes of cortical and pith parenchyma cells, diametrical growth of the cells begins to substantially exceed their longitudinal growth.

Simultaneous with these changes in the microtubers, the apical meristem continues producing leaves, but the activity slows down after the growth of two to four leaves; however the increase in the size of the cells bellow the meristem continues and expands to the periphery of the apical meristem of the tubers. In this condition, the meristem deepens and the last primordiums cover it in the form of protective scales (Fig. 8A-B). likewise, the apical and lateral buds can be observed as partially lodged into the microtuber tissue and covered by scaly leaves. The trend of the increase in the number of cells in the length and with of the tubers corresponds to the changes in the size of the tuber and stops when the tuber grows to the size of 7-8 mm.

## DISCUSSION

Potato microtubers grow out of stolons, which themselves have sub-branches and are usually located at the basal nodes of the plant. Tuberization begins with the longitudinal growth of the stolon turning into radial growth. Each fully grown tuber has a periderm consisting of phellem (suberose tissue) phelogen (phellem-producing cambium) and phelloderm (posterior cortical parenchyma tissue). The long cells of the cortical parenchyma tissue separate the peridermal tissue from the vascular ring and the pith (Desfontaines, 1998). The buds in the eyes are structurally similar to the buds in the apex of the branches.

Biochemical studies of Tuberization have revealed that the production of starch begins before tubers begin to grow. The levels of fructose, glucose and the neutral invertase enzyme are positively correlated with each other, which is indicative of the important role of the enzyme in the accumulation of reserve sugar in growing tubers.

Cell division and growth are both influential in the growth of tubers (Fujino, 1995; Gopal, 1998). It is not clear weather the initial development of stolon is caused be cell division or cell growth. Some researchers report that mitosis takes place before increase in the cize of the cells. Other observations show that the initial radial growth is the result of the diametric growth in the cells, therefore the sequence and chronology of cell division and cell growth are not clear yet. Studies of cell mitosis and growth are a

key to the understanding of the process of longitudinal growth of stolon to radial growth. The present study shows that the first signs of tuberization, largely simultaneous with induced meristems, are: A- growth of cortical parenchyma cells as a result of the development of its vacuoles, B- cell divisions in the perimedullar region and in the inner sections of the meristem, which are observable after the fourth day. During the growth of tuber, different types of tissues are involved in cell division and growth.

Cutter (1978) believes that Tuberization is acropetal and each tuber, regardless of its position on the plant, originates in the sub-apical region, particularly at the youngest growing internode. The initial stages of tuberization begins with the acropetal growth of the stem axis. Along with Tuberization, mitosis and apical meristem activity of the stolon slows down.

It was observed in the present study that when the apical meristem of the newly formed microtubers reduces its leaf forming activity after the second or the fourth leaf. Gopal (1998) that plants growing in permanent darkness begin Tuberization earlier and grow old sooner; the tuber of eyes in each microtuber also decreases in such conditions. The epidermal cells of the posterior perimordium and the initial pattern of the leaves begin differentiation and developing vacuoles earlier than the cells on the ventral surface; the same phenomenon is also observed in the formation of mesophilic cells. During the differentiation of epidermal cells, which is simultaneous with their growing longer, the growing vacuoles begin to move towards the outer septum of the cells and cover the nucleus as a protective structure. The review of literature, however, did not reveal any documented report in this regard. The opinion of other researchers about epidermal protective tissue and the origin of peridermal tissue indicates the fact that the epidermis of young tubers are uniseriate and have stomata, but this layer is quickly by the periderm. Phellogen, which produces peridermal phellem results from periclinial divisions in the epidermis (Hussey and Stacy, 1984). The epidermis of very young tubers is replaced by buberinazed phellem cells, themselves originating from the lateral meristem or phellogen. Phellogen originates among the epidermal cells. Apparently phellodermal tissue (posterior skin) does not grow out of phellogen (Xu and Veugdenhil, 1998). There is a contradiction between this view and the results of this study, which can be due to genotypic difference between different kinds, or differences between the in vivo and in vitro culture conditions. Peridermis acts as a protective layer to prevent dehydration of parenchyma cells of the specialized tuber and prevents the infiltration of different pathogenic elements from the soil.

Reeve (1969, 1970) observes that considering starch content, cortical parenchyma cells are more homogeneous and more evenly distributed than other types of parenchyma cells. Generally speaking, cortical parenchyma cells have more starch granules than pith parenchyma (Ruzin, 1999). Earlier study indicates a correspondence between cell and tuber growth (Reeve, 1973). The cause of the cessation of cell division in cortical parenchyma is the short-living activity of the inner cortical parenchyma (part of the preimedullary tissue), such that the traces are observable until the twelfth day after the initial formation of tubers. In line with this viewpoint, some researchers have commented that in spite of the importance effect of cortical tissues on the solon, but they do not grow very much in number during the growth of tubers, therefore secondary structures are found in fully grown tubers.

As mentioned, in growing tubers, the growth pattern in cortical and pith parenchyma cells and vacuole movement cause radial growth to exceed longitudinal growth. The studies of Fujino *et al*. (1995) in their studies in this regard observe that adding 8% saccharose to the culture causes the branches to stop growing and protrude in their sub-apical regions. The protrusion of this region begins with lateral growth and later with periclinal division. In the next stage cells begin to divide and begin their lateral growth. These changes coincide with rearrangement of superficial microtubers (MTs). In the cells of the sub-cortical region of growing branches, MTs have transversal arrangement. In growing cells MTs arrange themselves longitudinally along the shoot axial. On the other hand, lateral branches treated with GA3 grow longer and in the sub-apical region of such branches one can see cells with transverse MT arrangement; this arrangement does not change even at environments with more than 8% sacharose.

Cutter (1978) reviewing the distribution of cell division and growth mentioned in different papers, observes that there is not much agreement on the role of different tissues in the growth of tubers. Part of the problem stems from differing interpretations of its morphological and tissue ontology. Following the initial radial growth, considerable increase in the number and later, size of the cells causes the tubers to grow. The comparative effect of each of these parameters has interested many researchers, but one cannot see a unified understanding of the issue. As mentioned, some of the differences could be stemming from the variety in the kinds studied and some from measurement errors. There is, however, general agreement that changes in the parenchyma in the inner phloem (peripheral parenchyma) and less importantly, the parenchyma in the outer phloem

play a role in the growth of the tubers. In general, review of the related literature indicates that many researchers agree on the role played by cell division in cell and tuber growth. Xu *et al.* (1998) believe that the time and place of cell division and growth differ in different areas of the stolons and growing tubers. When protrusion begins in the apex of stolons, transversal cell division stops in the tip of the stolon.

Although longitudinal divisions do take place in the pith and cortical regions over a short period of time, researchers are of the general opinion that they are not small in number and contrary to what Reeve (1973) and his colleagues believe these cell divisions in the cortical and pith areas are always parallel to the longitudinal axis of the stolon and causes the transversal growth of the apical region in tubers. Although these divisions have been randomically observed in the perimedullary region in the initial stages, the main growth of the peripheral region includes both cell division and cell growth. The division pattern in the peripheral region is randomly arranged and causes the tuber to grow in all directions; therefore the perimedullary region constitutes the major part of the fully grown tuber.

Xu *et al.* (1998) believe that the morphology, cell division and cell growth of in vitro tubers are similar to the formation of in vivo tuberization, but in vitro conditions, tubers stop growing when they reach the size of 0.8 cm. it appears that the larger size of in vivo tubers is the result of cell division and later cell growth in the peripheral region, which is not true in the case of in vitro tubers. According to Yu *et al.* (2000), the overall density of sugar decreases regularly with the increase in the size of the tubers. During the sixth week, microtubers gradually cease to grow. Studying the density of sugar indicates that the density of sacharose drops from 80 to 6 g $L^{-1}$ during the first week. A large part of the sacharose converts into fructose and glucose. The overall density of sugar reaches 20 g $L^{-1}$ in the period between the second d week and the end of the sixth week; the hydrolysis of sacharose produces equal amounts of fructose and glucose. The density of glucose, however, is always lower than that of fructose, which means that potato absorbs glucose more efficiently than fructose.

There is apparently a positive relation between the growth of microtubers and changes in the sugars in the nutrient solution. This shows that one of the successful strategies to stop the premature growth of microtubers is to prevent or reduce the hydrolysis of sacharose, which increases the efficiency of sacharose use in the culture of potato microtubers.

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