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The Study of Anther Development, Microsporogenesis and Formation of Male Gametophyte in Potato (*Solanum tuberosum* L.)

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Abstract: Potato with scientific name of *Solanum tuberosum* L., belongs to the Solanaceae family and is an edible herbaceous plant which has medicinal properties. In this study the process of anther and pollen development in the potato was studed. Flowers and buds are harvested at different stages of development, fixed in FAA and were maintained in 70% alcohol. The samples, being prepared and embedded in paraffin were cross-sectioned with the microtome. Staining was performed by haematoxylin and eosin. The results showed that the plant has 4 bags of pollen per each anther, anther wall development in this plant is of the kind found in the dicotyledonous plants and is formed of a layer of epidermis, a endotheciumlayer, middle layer and a feeder layer (Tatetum). Its nourishing layer is secretory and simultaneous cytokinesis is used to separate tetrad. The microspore tetrads are of quadrilateral (tetrahedral) type. Pollen grains include 2 cells at the time they are released and have three vegetative splits.

Key words: Microsporogenesis, anther development, pollen grains, *Solanum tuberosum* L., FAA

INTRODUCTION

Floral development is a complex biological topic which starts with formation of a small mass of undifferentiated cells but it is organized to a complex structure in which different organs have occupied specific and exact positions. In addition, every organ has its own cell types organization and functioning (Meyerowitz *et al*., 1989).

Potato (*Solanum tuberosum* L.) of the Solanaceae family includes 147 genera and 2,930 species in the world (Judd *et al*., 2000). South America is the main origin of this plant. So, that some instances of that are still wildly grownin the northern parts of Chile and Argentina. These plants are commonly grown in all regions of the world today (Zargari, 1982).

Potato is an annual herbaceous plant with above-ground stem compound with 7-12 original leaflets. Smaller leaflets are also located among these leaflets which give a specific appearance to the plant, so that their existence makes it easy to distinguish the plant from other species of Solanum (Zargari, 1982).

Inflorescence is of the pistil kind in this plant, the flowers have five sepals, five continuous petals and 5 flags fused at the base and encompass the ovary. Flag poles are short and pollen bags are long. Potato's radius ovary is formed of two carpels with a long style and a double stigma. Ripe fruit is a berry and resembles a small green tomato. Flower color in different varieties ranges from white blue, blue-purple to reddish-violet (Rezai and Soltani, 1996).

Potato tubers contain 75% water, 22% carbohydrates, 0.99% of nitrogenous substances, 0.15% of fat substances and 0.09 of various mineral salts organic acids and different vitamins B1 and B6. Green organs such as leaf and stem of the plant have narcotic and tranquilizing effects due to Solaninein them and can be effective for elimination of neuralgia and rheumatism, dry cough and diarrhea with vexation but are not domestically used because of their toxicity. Solanine exists in the under-epidermis layers of potato and around its tuber sprouts. New sprouts of potato tubers contain about 0.04% of solanine and its toxicity symptoms include headache, dizziness, vomiting, cramping and severe poisoning leading to death (Zargari, 1982).

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The present study is the first report on the development of anthers and pollen of *Solanum tuberosum* L. species. According to the studies conducted on references there is no enough and precise information on the genetic structure of the anthers and pollen of the plant. Therefore such comprehensive research seems to be necessary and this research can be a good model for the plants in potato family.

MATERIALS AND METHODS

In order to investigate the formation of anthers and pollen, flowers and buds of the plants at different stages of development were harvested from early May to late August 2014 on a farm in the North West of Tabriz in Iran. The samples were stabilized in FAA stabilizers (37% formaldehyde-acetic acid glasyal-ethanol at a ratio of 17: 2: 1 mL) for 12-24 h. After washing the samples in running water, dehydration with increasing percentages of ethanol, clearing with increasing percentages of toluene, Paraffinization with a solution of increasing molten paraffin (60-62°C), embedding in the molten paraffin, provision of thin sections of 8-10 mm thickness with microtome were performed. The sections were glued on glass slides using Hapt glue, paraffin was removed with toluene, watering was performed with decreasing degrees of ethanol, staining was done with H and E, final clearing was done by placing slides in toluene and finally the final assembling (pasting the lamella) was done using entellan glue. The development stages of flags were investigated in consecutive sections by Zeiss Axiostar plus optical microscope in different magnification. At least 10 flowers were sectioned for each stage. And the best sample was photographed by a digital camera (Yeung, 1983).

RESULTS AND DISCUSSION

The flower structure includes four rings: 5 sepals, five petals, 5 Flags fused at the base 5 and include 5 anthers with four pollen sacs (Fig. 1f). After preparing the cross-sections of the young and mature anthers of potato the following results were obtained: anther has four pollen bags (Tetrasporangal) (Fig. 1a, b). In the early stages of anther development 5-8 rows of cells are differentiated under the epidermis that produces the spore-bearing tissue towards the inside and parietal layers towards the outside. Followed by mitotic divisions in the mass ofspore-bearing cells these cells are directly differentiated into microsporocytes or microspore mother cells. Microsporocyteswith dense cytoplasm, large size and large nuclei of cellsare differentiated from the Adjacent cells (Fig. 1i).

Anther wall consists of four layers which include the epidermis, endothecium layer, middle layer and layer of nutrients (Tapetum) from the outside to the inside. Epidermis is made of elongated and flattened cells that protect the developing anthers. The temporary layer is located after the endothecium layers in this species this layer is thin and includes stretched and narrow cells. Anther innermost wall layer is Teptum or the nutrient layer that isoften single-core orhas underwent mitotic division and is dual core. These cells encompass the spore-bearing tissue of anther these cells in the fall. Teptum has important role in providing food for the developing pollen (Fig. 1g).

Once the meiosis begins around Microsporocytes the special walls start to form (Fig. 1h). With Meiosis I in pollen mother cells, two n chromosome Dyad cells (Fig. 1k) and with Meiosis II four n chromosome cells (haploid) that are known as Tetraspor are formed. The Tetrads are quadrilateral (Tetrahedral) (Fig. 1l). No wall is formed between the nuclei of the Telophase I and Simultaneous cytokinesis occurs after Telophase II. The special wall amidst the monads and around tetrad is clearly recognizable (Fig. 1j) with dwindling of the special wall, the cells forming the tetrads are come apart and Microspores are released. Microspores are not vacuoles when they are released and have a dense cytoplasm, a regular form and adistinctive nucleus located in the center of the cell (Fig. 1m). With the development of the central vacuole, the nucleus of the cell is driven to the side of the cell (Fig. 1n).

Then the nucleus divides by mitosis and two unequal nuclei are created. A great vegetative nucleus and a small reproductive nucleus which leads to formation of dual core pollen and ultimately a double-cellpollen (Fig. 1o).

In mature anthers, before flourishing, two bags of pollen bind together in each half anther and form the pollen house (Fig. 1d). With development of pollens, anther walls are also changed. The middle layer is dwindled in the early stages of pollen development and the Tapetum-layer cells are almost dwindled at the single cell pollen but their remains are still visible. When microspores are developing into mature pollens some annexes of these cells are formed towards anther cavity and the Tapetum layer appears to be of secretive kind. Pollen grains are almost spherical andtricolporate. With completion of the development of pollen grains in the anther wall, only the epidermis and endothecium layers remain which make the anther bloom when it is mature (Fig. 1e).

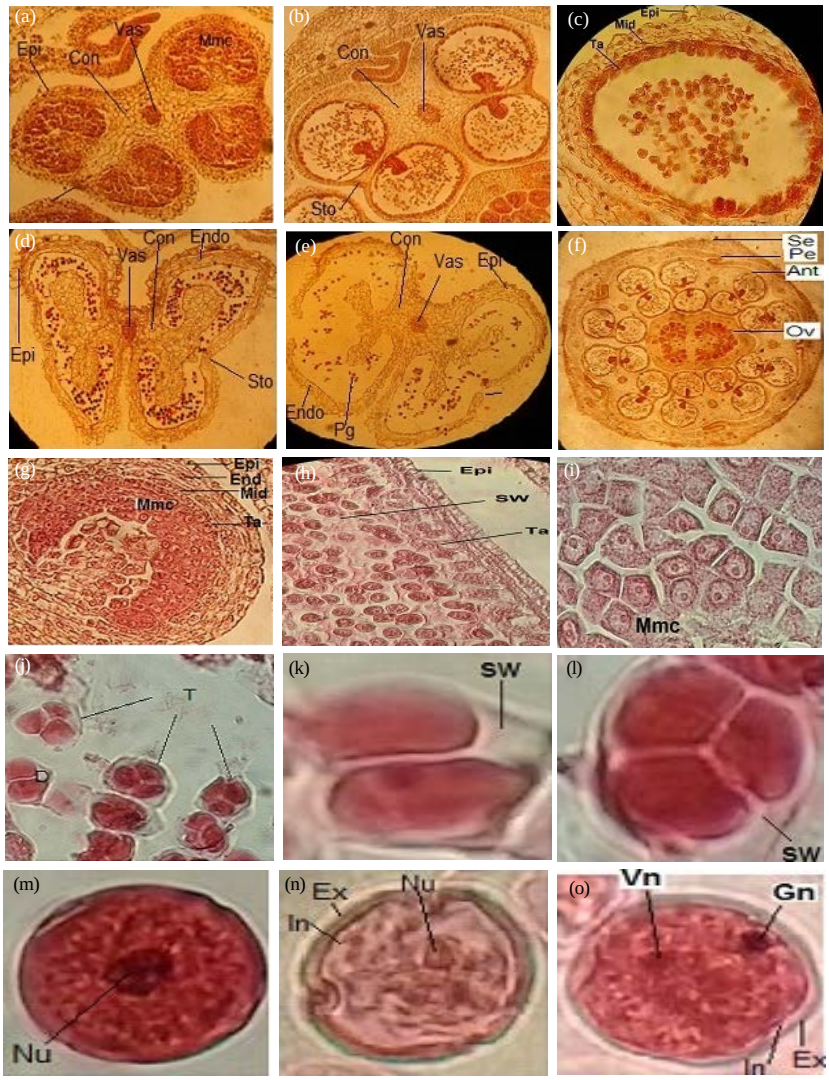

Fig. 1: Microsporogenesis, formation of the male gametophyte and development of Anther wall in (*Solanumtuberosum* L.): a) cross-section of Anther which shows four developing sacs of pollen; b) tetrasporange half-mature Anther that is seen in the lips; c) a view of a pollen sacs; d) cross-section of mature anthers before blooming, binding of the two bags in each half of anthers and formation of pollen house; e) opening of anthers and release of mature pollen grains out of that and elimination of the lips interface; f) cross-section of a flower shows five anthers with four sacs of flower pollen. Ant: Anther, Con: Connective, Endo: Endothecium, Epi: Epiderm, Mid: Middle layer, Mmc: Microspore mother cell, Ov: Ovary, Pe: Petal, Pg: Pollen grains, Se: Sepal, Sto: Stomium, Ta: Tapetum, Vas: Vascular strand; g) four layers of anther wall are recognizable: the Epidermis (Epi), Endothecium layer (End), the Middle layer (Mid) and Tapetum layer (Ta); h) longitudinal section of anther walls that shows the Special Wall (SW); i) microsporocytes or microspore mother cell (PMC) with dense cytoplasm, large size and distinctive cells are differentiated from of the surrounding tissue cells; j) asynchronous division at the anther's pollen bag, Dyad presence and simultaneous division of the cytoplasm and the formation of tetrad; k) Dyad stage (D) and Special Wall (SW) around it; l) Tetrad stage (T) and Special Wall (SW) around it; m) the microspores are in regular form and their single nucleus is visible; n) mature pollen with Exine (Ex), Intin (In) and Nuclei (Nu); o) dual nucleus Pollen, large Vegetative nucleus (Vn) and a dense Generative nucleus (Gn)

CONCLUSION

Microscopic observation of flower bud and their longitudinal section in different developmental stages show that in the examined species, the four-layer anther wall development is done on the basis of dicotyledontype and consists of the epidermis, endothecium, temporary and Teptumlayers (Davis, 1966).

Archesporium cells with dense cytoplasm and large nuclei are recognizable. The cells are tangentially divided and form the inner parietal cells and the outer spore-bearing cells (Xue and Li, 2005). Meiosis is every microsporocyte leads to the formation of tetrads. Tetrads are of the tetrahedral type.

This observation is similar to Lebon *et al*. (2008). But is not correlated to Chehregani *et al*. (2011) studies that reported the presence of four-corner (tetragonal) and linear tetrads in cornflower and four-corner (tetragonal) tetrads in chicory.

There is a clear correlation between meiosis in pollen mother cells and anther development of anther Tapetumlayers which has also been reported for other species of the family (Gustafzsson, 1946).

Cytoplasm division in created by the pollen mother cells meiosis is of the synchronous type which is in conformity with the findings by Deng *et al*. (2010), Pacini and Keijzer (1989) and Majd *et al*. (2014).

Microspores have no vacuoles when released from tetrads and have a dense cytoplasm, a regular shape with a large nucleus located in the middle of them. Mature Microspores undergo mitosis and each one of them evolves into young pollen that has a large vegetative nucleus and a smaller generative nucleus. Therefore, first a dual nucleus pollen and then a dual-cell pollen is formed. These observations are in conformity with Chehregani and Sedaghat (2009) and Jafari and Niknam (2012) reports. The results by Sood and Kumar (2000) studies showed that pollen grains of this genus are in the tricellular maturity stages which are not in conformity with our findings.

The Tapetum layer in the examined plants is of secretory type which is in conformity with findings by Jafari and Niknam (2012) who described the nutrient layer as secretory.

Tapetum layer cells show a high degree of ploidythet represents their high metabolic activity and in this respect they are similar to the Anti-fabric cells of the embryosac (Maheswari, 1950).

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