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Anther development and microsporegenesis in *Gossypium herbaceum L.*

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ABSTRACT

Gosseypium herbaceum L. is known as cotton plant belongs to the family Malvaceae. This plant is widely distributed throughout western India, Africa, Middle East countries, central Asia, and Availability is found in Iran, Afghanistan, Russian and Turkistan. Gossypium herbaceum L. is a cultivateda cotton species (2n=2x =26,A_1 A_1) has favorable traits such as excellent drought tolerance and resistance to sucking insects and leaf curl virus. In this study, microsporegenesis and anther development of G.herbaceum were studied. The flower, in different developmental stages, were removed, fixed in formalin-glacial acetic acid-alcohol(FAA),stored in 70%ethanol,embedded in paraffin and then sliced at 8-10Mm by rotary microtom. Staining was carried out by periodic Acid Shiff (PAS)and contrasted with hematoxylin. Scanning electron microscope(SEM) was used to analyze the mature pollen grains. The results indicated that anthers wall development followed the dicotyledonous type and were tetrasporangiate which composed of epidermal layer, endotheciumlayer, middle layer and tapetum layer.

Key words: *Gossypium herbaceum*, microsporo genesis, anther development

INTRODUCTION

Floral development is a complex biological topic which starts with formation of a small mass of undifferentiated cells but is organized to a complex structure in which different organs have occupied specific and exact positions. In addition every organ has its own cell type's organization and functioning. [18].Cotton is the most important natural source of fiber currently used in the textile industry.

It is also a valuable source of oil and margarine with cultivated species of cotton containing 14.6-25.6% oil on dry seed weight basis (pandey, 1976).cotton seed meal contains high percentage of protein, and is an important source of essential amino acids like lysine, methionine, and tryptophan (EL-Nocrashy et al., 1969).

Malvaceae is family of flowering plants containing 243genera and at least 4225 species of herbs , shrubs , and trees. Economically, the most important member of the family is Gossypium (cotton).[23].the genus Gossypium comprises 50species including cultivated cotton i.e. two diploids (*G.arboreum* ,*G.herbaceum*)and two tetrapolpids (*G.hirsutum* , *G.barbadense)*.[23]. The endemic cotton in Iran is of herbaceum variety which has relative resistance to lack of water, moistority, and has resistance to some pests and disease.

G.herbaceum plant is hairy plant,2-8 feet high with thick woody stem and leaves sparsely hairy, rarely glabrous. The leaves are 5-7 lobed .The flowers are yellow with purple center Development of anther involves a series of occurrences to produce and release mature pollen grain from anther. The aim of this research was to investigate a detailed study development of anther and microspore genesis of G.herbaceum and evaluation of pollen grain ornaments by scanning electron microscope(SEM).

MATERIALS AND METHODS

Flowers at all different developmental stages (tiny buds to mature flowers).

Were collected and fixed in FAA solution(20%formalin, 10% acetic acid, 70%ethanol v/v).

Dehydrated during alcohol series. embedded in paraffin and sectioned with a thickness of 8-10mm using rotary microtome. Staining was carried out with PAS (Periodic Acid Schiff)technique according to protocol suggested by yeung and contrasted with Hematoxylin. Anther development investigated with a Zeiss Axiostar plus lightmicroscope. Many samples were studied before each stage and photomicrographs were made from the most effective ones.

Palynological studies:
In order to study pollen grains, photographs were taken using SEM microscopy, made of Kyky company model EM3200. Firstly, pollen grains were dehydrated. Then they were mounted on aluminum stubs and covered with a layer of gold and considered with electronic microscopy equipped with computer.

RESULTS

Results obtained from this study demonstrated that each young anther was consisted of4 pollen sacs with connective tissue in the center (fig 1) . Each pollen sac was concluded of peripheral cells forming an undifferentiated wall. Then wall differentiated to epidermis, endothecium, middle layer and tapetum layer (fig 2). At next stage microspore mother cells were also detectable .microsporocyte are recognizable by their large volume, dense cytoplasm and conspicuous nuclei. Each microspore mother cell undergoes meiosis, during which MMC undergo successive type .In this type of cytokinesis each nuclear division was followed by cell wall formation. The first nuclear division of meiosis (meiosis1) is accompanied with the formation of two haploid cells in a dyad form (fig3 A, B).Then the second meiotic division occurs in dyadic cells (meiosis2) which was followed by wall formation and ultimately tetragonal tetrad (Fig4A, B) which was enclosed with special callosic walls at the stage of tetrad the anther wall was composed of epidermis, endothecium and tapetum (Fig2). This means that the middle layer was degenerated.

At next stage haploid cells were segmented after decomposition of the special wall .and each of the meiospores is turned in to young meiospores with big and central vacuoles and small peripheral nucleus. Exine evolved in this stage, at the stage of anther dehiscence. (Fig6) .Additionally we found the miotic division of microsporeunequal. Therefore, adarker generative nucleus and a lighter vegetative nucleus were detectable(Fig8).Exine layer of pollen grains seen well in SEM graphs.(Fig9,10).

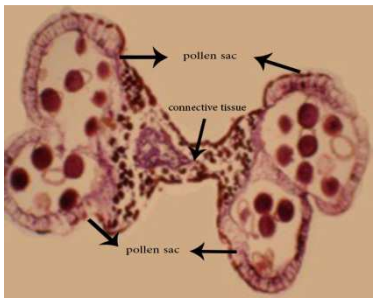


Fig1:pollen sacs with connective tissue

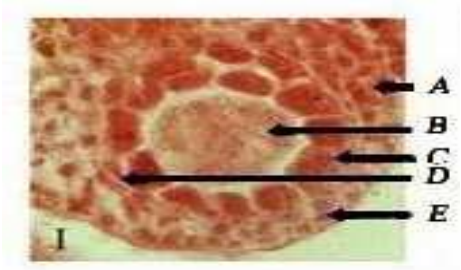


fig2:micrograph of pollen mother cell of the early anther
A:epidermis,B:pollen mother cell, C:tapetum, D:middle layer, E: fibrous layer

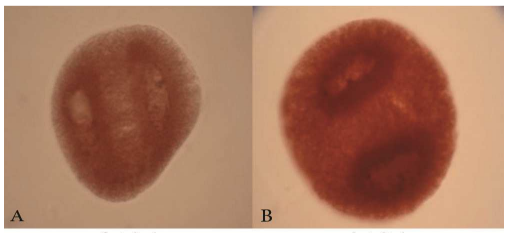


Fig3:diad

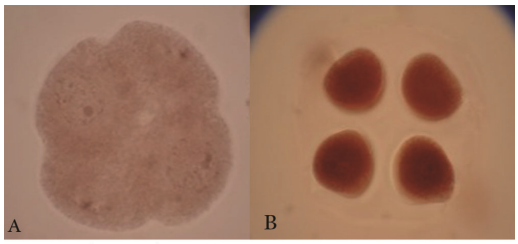


Fig4:tetrad

DISCUSSION

Results obtained from this study revealed that developmental stages of anther in *Gossypiumher baceum* L. Anther wall followed dicotyledonous type which was composed of an epidermal layer, anendotheciallayer, middle layer and tapetum layer that was similar with the finding of Chehreganietal 2009 for *lepidiumvesicarnum*L.There was a significant correlation between microspore mother cell divisions and anther development that is confides with other reports for dicotyledonous plants(Davis1966).Meiosis of every microsporocyte leads to the formation of tetrad. this observation is similar to(lebon et al2008).The first nuclear division of meiosis (meiosis1)is accompanied with the formation of two haploid cells in a dyad form, that was similar with the finding of Bagheri Abyaneh etal 2014 for*lepidiumsativum*. There is a clear correlation between meiosis in pollen mother cells and anther development of anther Tapetum layers which has also been reported for other species.(Gustafzsson,1946).

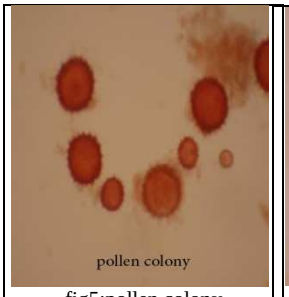


fig5:pollen colony

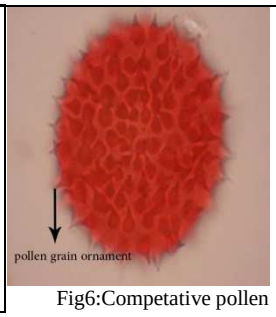


Fig6:Competative pollen

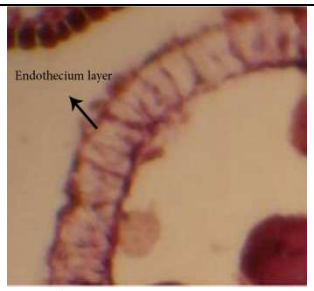


fig7:Endothecium layer

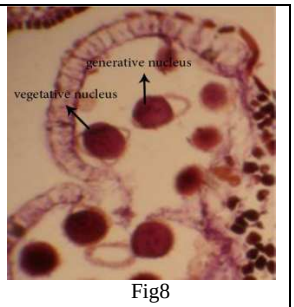
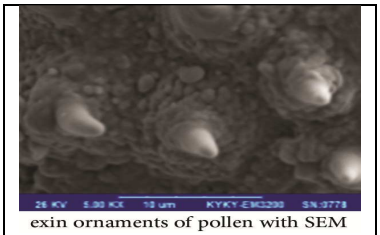


Fig8

sem micrographs pollen of G.herbaceum

fig9



exin ornaments of pollen with SEM

fig10

Second meiotic division occur in dyadic cells (meiosis2) which was followed by wall formation and ultimately tetragonal tetrad.(Bagheri Abyaneh etal 2014 for *lepidiumsativum*).

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