

Male gametophyte development steps in *Pistacia vera* L.

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Abstract. Sadeghirad E, Majd A, Iranbakhsh A, Javanshah A. 2018. Male gametophyte development steps in *Pistacia vera* L. *Nusantara Bioscience* 10: 151-158. Salinity affects the growth and development of plant. It also affects the development steps of male gametophyte in pistachio plant. Pistachio (*Pistachio vera*) is a member of family Anacardiaceae and order Sapindales. To study the effects of salinity on those steps, an experiment was performed in two locations in Golshan Anar with commensurate circumstances, namely: a control area (A) which was well-irrigated with fresh water, and the other area (B) which was well-irrigated with salty water added with NaCl solution with EC values of 14 dS.m⁻¹. The flowers sampling was done in two Golshan Anar regions on the springtime based on a completely randomized design with three replications. The development steps of male gametophyte in pistachio plant were observed using conventional cell histology techniques and light microscopy observations and were then contrasted with samples subjected to no salinity stress. The results represented that several steps of male gametophyte development are as follows: (1) the anther experiencing normal growth which is tetrasporangiate, (2) cytokinesis taking place simultaneously with meiosis in the microspore mother cell, the tetrahedral tetrads, (3) microspores being delivered after meiosis by microsporogenesis were more or less irregular in shape during the contraction period. Finally, the abnormal shape and structure of the number of cases reviewed in three replicates of pollens can be one of the significant factors influencing the decline in the product.

Keywords: Anther, *Pistacia*, salinity

INTRODUCTION

Adversely, salinity influences the growth and development of plant and it is a major environmental stress limiting agricultural production (Saini 1997). Reproductive outgrowth starting from meiosis in the spore mother cells for fertilization and untimely seed formation is highly sensitive to various stresses, such as drought, heat, cold, flooding, and nutrient insufficiencies (Salter and Goode, 1967; O'Toole and Moya, 1981; Saini and Aspinall, 1981, 1982; Westgate and Boyer 1986; Satake and Yoshida 1978; Schoper et al. 1987; Morrison 1993; Hayase et al. 1969; Brooking 1976; Matsushima, 1962; Zavadskaya and Skazkin 1960; Campbell and Leyshon, 1980; Sharma et al. 1987; Azouaou and Souvré 1993; Lardon and Triboi-Blondel, 1994). These stresses lead to several structural and functional deformities in reproductive organs, causing failing of fertilization or premature abortion of seed or fruit. Thus, the breakage to fecundity from stress at this phase is notably critical for crops in which the economic outcome is the result of sexual prolificacy, as elevation in water shortage possibly characterizes as the most significant environmental element restricting global crop fecundity (Fischer and Turner, 1978; Boyer, 1982). Among these stresses, salinity is a main question since the past centuries. Around 20% of land under implantation and nearly 50% of watered lands in the world are influenced by ion concentration (Zhu 2002). Elevated salinity has two spoiling impacts on plants. The first is the impact induced by the water deficiency as a product of escalating

concentration of soil solution and the other is the impact of toxic ions which hinder enzymatic activity in key processes (Zhang and Blumwald, 2001).

Plants utilize various mechanisms to reduce the impacts of these environmental stresses. One of these mechanisms is the imperfect establishment of eggs or pollen that leads to the displacement of the food founts from the reproductive organs into metabolic reactions and leads to stress resistance. The aging of the reproductive organs can also be stimulated or predated by salinity stress. Asch and Wopereis (2001) stated that 45% of the rice harvest is lessened by salinity stress as an impact of the clusters sterility and the seed weight reduction in formed seeds. Salinity becomes a major cause of defective grain and product and quality reduction in cotton (Davidonis, Johnson, and Landivar, 2000).

The studies of Namuco and O'Toole (1986) and Westgate and Boyer (1986) indicated that during plant growth microspores have elevated sensitivity to salinity. To the mind of Sun et al. (2004), on salinity stress conditions, microsporocytes of *Arabidopsis* plants did not grow into mature pollens grains but these cells became vacuole and got old in two days. Moreover, the remaining pollen grains stay in the fallen anthers. Prolonged periods of salinity stress gave no effect on mature pollen grains. It verifies that the effect of salinity on the pollen grains relies on the developmental phases of anthers. Male reproductive perfection in plants is exceptionally susceptible to water deficiency during meiosis in the microspore mother cells (Saini 1997). Pistachio (*Pistachio vera*) a member of the

family Anacardiaceae and order Sapindales (Al-Saghir, 2010). Morphologically, pistachio (*P. vera*) is recognized as the oldest species of this genus (Baninasab and Mobli, 2008). *P. vera* is a dioecious woody plant with imparipinnate leaves which fall in autumn (Al-Saghir, 2010).

The male and female anthesis have 450-500 and 150-250 flowers, successively. A very long life is owned by this tree whose height can reach 7-10 meters (Asaja, 2006). The surface of the stigma receives pollen when the female flowers are exposed, then pollination takes place that will produce fruit and seeds. The female flower buds located on the branches of a one-year-old tree begin to bloom in late March and 100 to 300 flowers are pollinated in every inflorescence during the first two weeks of April (Polito and Kallsen, 2005). Researchers have conducted many different studies on this species including morphological and developmental studies on female flowers and embryogenesis of the genus *Pistacia* L. (Al-Saghir, 2010; Bachelier and Endress, 2007; Grundwag, 1976) and species *P. vera* (Lin et al. 1984; Martinez-Palle and Herrero, 1998; Shuraki and Sedgley, 1996; Shuraki and Sedgley, 1997), Endress and Bachelier (2007) have examined the genus *Pistacia* L. and reported that female flowers have 5-8 excrescences similar to calyx, the female portion holds a large round ovary with short vigor and trifurcation of stigma (two lobes on each branch). Grundwag (1976) stated that this genus has one ovule per ovary, a downward-moving ovary, a monolayer, and many nuclei. The embryonic sac of the genus (Grundwag, 1976) and species (Lin et al. 1984) have Polygonum-type. Yet, there are various studies on male flowers (Al-Saghir, 2010; Asaja, 2006; Azouaou and Souv  r, 1993; Bachelier and Endress, 2007; Shiyan et al. 2001; Li et al. 2011; Qiu et al. 2010). Various researches have been done on the morphological structure, but less attention has been provided to the founding of sapidity in pistachios. Other researchers carried out morphological studies on pistachio pollen and declared that their morphology was diversified among different varieties (Afshari et al. 2008; Davarynejad et al. 1996; Li et al. 2011; Qiu et al. 2010).

Pistachio beans own a special economic significance in the family Anacardiaceae, genus *Pistacia* L. in accordance with Molecular Data Bank of Iranian Pistachio reported in 2008, pistachio is the first non-oil exported product in Iran (IPMD). Several techniques and studies have been carried out to establish the knowledge about this precious species as well as to determine distinction among diverse varieties. Yet, the morphological and developmental evaluation of flowers, particularly male flowers, are very finite. The essential characteristics of reproductive organs and understanding the developmental phases of gametophytes find a substantial significance in botanical sciences since they are the proper equipment for the recognition and categorization of plants. The hodiernal inquiry arranges to learn anatomical characteristics of male flower and developmental phases of anthers in pistachios and the impact of salinity on these phases in natural circumstance with no entry of any natural and experimental factors. Biological stress of *Pistacia* was observed by Seydi et al. (2015), Parsa and Karimian (1975), Ranjbar et al. (2002),

Chelli-Chaabouni et al. (2010), and Bastam et al. (2013).

MATERIALS AND METHODS

This comparative study was carried out on two locals with identical conditions involving control garden (A) which was watered with fresh well water and other garden (B) which was watered with salty water with EC (electric conductivity) value of 14 dS.m⁻¹ NaCl solution. Based on a completely randomized design with three replications, a sampling of flowers was taken place in two gardens of Golshan Anar area in the springtime. Golshan is a village located around Anar in the suburb of Rafsanjan city, Kerman Province. The study area is 14 km southeast of Anar, at 30°48'N and 55°21'W, and the average height of 1408 m with a desert and hot-dry climate. The sampling of soils was conducted on the surface up to a depth of 120 cm, it was to determine the soil texture, and, by this, the percentage of each of the three particles i.e. sand, silt, and clay were analyzed in the soil laboratory of Rafsanjan's Pistachio Research Center. Then, by the technique of soil textural triangle, the percentage of sand was determined as shown in table 1 (Mohammadi, 2006).

To learn about the developmental phases of anthers, sampling from male inflorescence buds was carried out until the opening of anthers and pollination. Morphology of inflorescence and male flowers was examined using a dissecting microscope. To learn about the anatomy and the development, the samples were soaked in FAA solution (90% ethanol 70 + 5% acetic acid+ 5% formaldehyde) for 24-72 hours (Johansen, 1940), and then placed in the running water for 24 hours. The samples were dried in ethanol with series of increasing concentrations and then soaked in alcohol 70%. For paraffin embedding, the samples were first dried using ethanol 70, and then the ethanol in tissues was little by little substituted with toluene (paraffin solvent) by soaking the samples in solutions with increasing toluene level for 20 minutes. Next, molding was carried out on samples by putting them in molten paraffin for at least 7 hours. Sectioning was performed using a rotational microtome with a thickness of 0.8 micrometers. The slides were deparaffinized by toluene and blemished with Hematoxylin-Eosin according to the protocol suggested by Meyer (Yeung 1984). To cling the samples, the sections were first dried in distilled water and increasing ethanol series and until they became transparent in toluene. Finally, the permanent slides were acquired using Entalen glue and coverslip. Microscopic examination of samples was performed under a light microscope (Olympus BH2 Japan) and the suitable samples were photographed with a digital camera (Canon IXUS 120 IS USA).

Table 1. Soil particles percentage and type of soil texture

Area of Pistachio	Soil particles			
	Type of soil texture	Clay (%)	Silt (%)	Sand (%)
A	Sandy loam (light)	19.8	25.6	54.6
B	Sandy loam (light)	19.8	17.6	62.6

RESULTS AND DISCUSSION

Results

The study on morphology and anatomy of male flowers indicated that the formation of male inflorescences is in the form of complex and lateral panicle on the branches before the leaves appeared (Figure 1.A). During the flowering

period, the color of anthers is red and it later changes during development phases. At the pollination period, they are completely yellow or their pedicel is yellow, but the tip remains red. The color of anthers changes starting from the ventral surface of inflorescence to the dorsal surface (Figure 1.B-C).

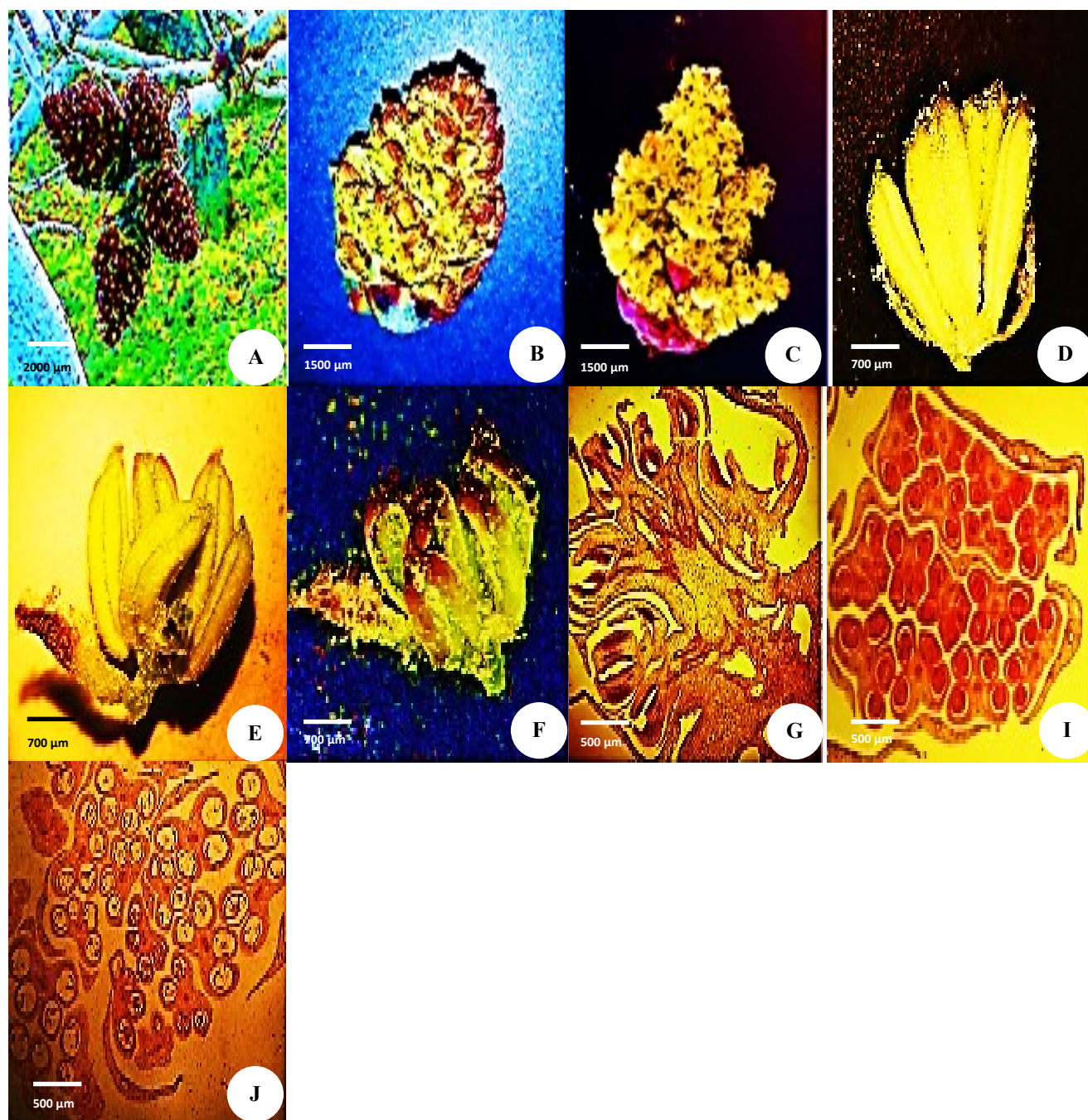


Figure 1.A-I. Morphological and anatomical structure of inflorescence and male pistachio flowers (*P. vera*): A. Emerging male inflorescence laterally before the leaves; B. Dorsal surface of the male inflorescence bridge along with red anthers; C. Surface of male inflorescence showing color changes of anthers to yellow; D-E. Male flower panicles with a short peduncle and large anthers with 2 sepal 1 bract; F. Flower with of dehiscent anther, G. Longitudinal section of inflorescence, the arrow refers to the secretory ducts in the side of vascular bundles; H-I. Cross-section of inflorescence, narrow arrow points flower with five anthers, thick arrow shows flower with four anthers and five anther arrowhead shows flower with six anthers and seven anthers, A = Anther, S = Sepal, B = Bract

Four to six stamens with large and bulky anthers are owned by male flowers, plus two or three sepals and 0-1 bract; the sepals and the bracts are recognizable by the size. Filaments are short and huge. In length and depth close to the central vascular bundle of anthers, the dehiscence of anthers occurs (Figure 1.D-F). The number of anthers per flower was confirmed by cross sections of male inflorescence and it resulted that the four-anther was dominant (Figure 1.H-I). The arrangement of flowers as panicle is confirmed by longitudinal section of inflorescence. In addition, the secretory ducts in the vascular bundles are exposed (Figure 1.G).

Three steps, namely, pre-meiotic, meiosis and post-meiotic involves in the development of anther and they are concluded from the examination of microscopic sections.

From division and differentiation of one or more epidermal anthers cell (s), microsporangium wall and spore-forming tissue are produced. By dividing high colorable cells and dense cytoplasm, spore-forming tissue is detected (Figure 2.A). The distance of two dorsal sporangia is shorter than that of the two ventral sporangia; at this stage, microsporangia located in a theca are far away from each other, and they are also split up from each other by septum in depth. As the spore-forming tissue divisions process occur, tangential divisions of some cells obtained from epidermal cell division take place to form the anthers walls. Pre-meiotic stage is finished by halting mitosis of spore-forming tissue and transformation of spore-forming cells to microspore mother cells that are large cells with bulk nucleus and dense cytoplasm (Figure 2.B).

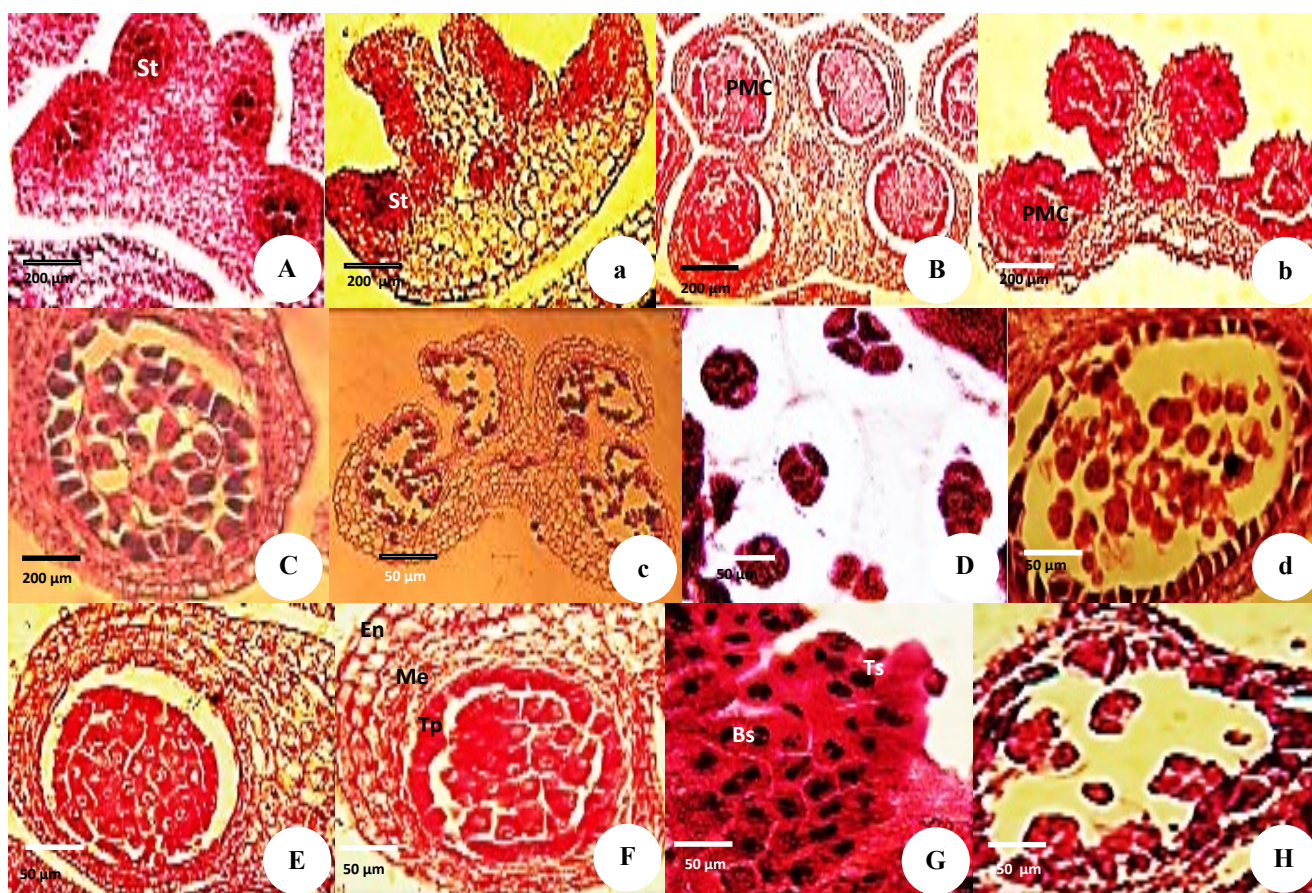


Figure 2. A-H. Stages of forming tetrad microspores inside of anthers in normally grown pistachio (*P. vera*) and in plants under NaCl treatment: A. Entire cross-section view of anther at the mitosis division of spore-forming tissue cells phase, in plants under salinity treatment on the right figure anthers were small, shriveled; B. Stopping of mitosis in spore-forming tissue cells and microspore mother cells, in plants under salinity treatment on the right figure anthers were small, shriveled and distancing from nutritional layer; E. Spacing between nutritional layer and microspore mother cells with high magnification; C. On the right figure entire view of anthers with microspores mother cells in meiosis; F. Anther wall of microspore mother cell meiosis time, which is composed of the epidermis, a mechanical layer, more than three middle layers and taptom; G. PMC in two and four-core stage, D&H. Cytoplasm division simultaneously in the microspore mother cells and production of tetrahedral tetrad surrounded by a thick layer of transparent callus, ST = spore-forming tissue, PMC = microspore mother cells, Ts = quad-core microspore mother cells (tetrad cell), Bs = a dual-core microspore mother cells (dyad cell), TP = nutritional layer

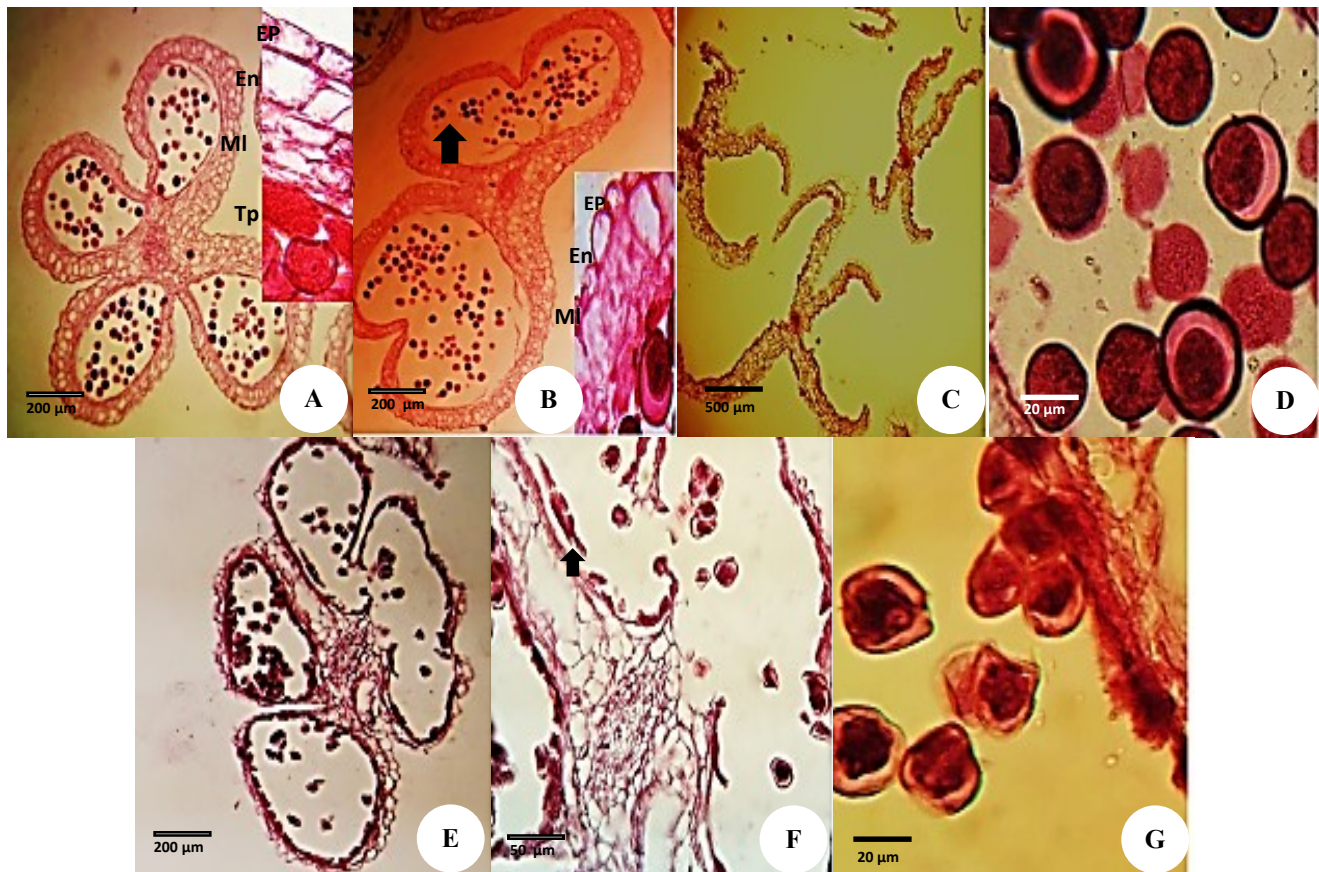


Figure 3. A-D. Anatomical structure of anther cross-sections of normally grown pistachio (*P. vera*), and E-G. in plants under salinity treatment since the production of free microspore to flourish anther: A. Complete decomposition of callus wall surrounding the tetrad and production of free microspores entire. Cross-section view of anther in the mature pollen. Arrowhead points out to the break down of the walls of a cavity for anther dehiscence. Anthers wall is composed of an epidermis layer, a mechanical wall layer, three middle layers and a tapetum layer, and entire view of anther cross-section at free microspores stage and disappearance of nutritional layer; B. Microspores feeding nutrition layer, leading disappearance of nutritional layers with the middle layer cells; C. Entire view of dehiscent anther; D. Mature pollen; E. Entire cross-section view of anther in the mature pollen grains. Arrowhead points out to break down the walls of a cavity for anther dehiscence in plants under salinity treatment, F. Anther with mature pollen Arrowhead points out to break down the walls of a cavity for anther dehiscence in plants under salinity treatment, G. Mature pollen under salinity treatment; EP = epidermis, En = mechanical layers, ML = middle layer

To begin the Meiotic stage was by taking away of the microspore mother cells from tapetum cells. At this stage, central indentation of each anther theca is completely deep and reaches to the central vascular bundle near to anther, so that the two microsporangia in the theca are completely separated from each other and the septum is hidden (Figure 2.B). As the phase of meiosis I happen, pectocellulosic wall of microspore mother cells is hydrolyzed and replaced by new callus walls. These walls keep away microspore mother cells from interacting with each other during meiosis. At the time of meiosis phase of microspore mother cell, anther wall is composed of epidermis, a mechanical layer, more than three middle layers and nutritional layer (Figure 2.F). In plants under salinity treatment, although florets appeared usual, but the growth of the anthers was unusual, and more anthers were small and whitered (Figure 2.A-D right). During meiosis of these cells, Cytoplasm cleavage happens at the same time. Inside of each

microsporangium at this stage, microspore mother cells with two and four cores are available successively after meiosis I and II (Figure 2.G). After meiosis, the cytoplasm cleavage is performed by the setting up channels of the microspore mother cell in which these cells are oriented as tetrahedral type and are called tetrahedral tetrad. All tetrads are of tetrahedral type and four cells are situated in a common callus wall and also are divided by a callus (Figure 2.H). Post-meiotic phase starts after the splintering of the callus wall and passing from tetrad phase to free microspore phase. At the commencement of this process, microspore is still in tetrad configuration. Microspores hold a certain nucleus with marginal place and congested cytoplasm after being exempted from tetrad at the beginning of discrepancy. Then, the callus lid vanishes entirely and the anther wall possesses mechanical layer, three middle layers and nutritional layer (Figure 3.A). At last,, middle and tapetum layers vanish with the

discrepancy of fully grown pollen grains; tapetum is glandular (Figure 3.B). Then the impact of condensing of mechanical layer walls become clearly visible, except the epidermis wall that causes to the impression of a U-shaped motif layer (Figure 3.D). Fully grown anther wall owns the mechanical layer with U-shaped motif along with the traces of tapetum layer and the middle layer. The separation of the two middle walls of each sac with the central part of anther causes to dehiscence in length and depth (Figure 3.A-C, E). Anther was shriveled and pollen grains owned unusual form (Figure 3.E-G).

Discussion

Pistachio is a plant with high economic value and it is native to Iran, as plant in cultivation, it is widely planted in various locations. It is so significant to do research on the bioecological and ecological issues of the plant, including the sorts of stress in Iran, such as the NaCl stress which is one of the major issues in cities and in the locations for pistachio cultivation. Biological stress of *Pistacia* was observed by Parsa and Karimian (1975), Ranjbar et al. (2002), Chelli-Chaabouni et al. (2010), Bastam et al. (2013), and Seydi et al. (2015). Al-Saghir (2010) in the study of the genus *Pistacia* L. informed that the flowers are small, monoecious, with no petal and panicle type of inflorescences. This research also informed that the flowers are monoecious and are born from female pedicel in complex panicle and on separate pedicels, as well as hold 4-6 stamens, 2 or 3 sepals and the 0-1 bract. The sum of stamens in the genus *Pistacia* were 4-6 pcs which has been informed by Hormaza and Polito (1996), 3-5 pcs by Shiyan (2001), and 4-5 pcs in species *P. chinensis* by Qiu et al. (2010). Al-Saghir (2010) in the research of genus *Pistacia* L. exerted bracteole term for non-bracteal excrescences surrounding flowers. Male and female flowers of this genus hold 1-3 small bracts and 2-7 bracteoles. Moreover, Bachelier and Endress (2007) familiarized non-bracteal excrescences as sepals and informed its numbers in male flowers of *P. lentiscus* are identical with their stamens numbers, which was 4-6. These researchers informed that they feel doubt if these excrescences are sepals or bract, they also informed that 5-10 sepals-like organs in *P. terbinthus* and only two of them in *P. mexicana*. In addition, as the flowers of the genus *Pistacia* are pollinated by wind, it indicates the tendency of evolution route towards being dioecious and losing perianth (petals shortage and diminished sepals). In our review studies, only a few numbers of reports were discovered about growth phases of anther to deliver pollen in the genus pistachios. there was individual study in the *P. vera* species done by Li et al. (2011). In this study, during meiosis, the anther wall of microspore mother cell consists of the epidermis, a mechanical layer with more than three middle layers, and a glandular tapetum layer. The stability feature of middle layers is important in this species. Because in most crops, middle layers vanish at untimely phase and before the establishment of the tapetum layer (Sanders et al. 1999), but in this species, middle layers vanish within tapetum layer at the pollen grains discrepancy, and this is in agreement with Li et al. (2011) who have also notified

this characteristic. The cytoplasmic classification was synchronous after meiosis of microspore mother cell, conformable to that of the polygonum type; this finding and glandular tapetum layer are in line with results of Li et al. (2011) on *P. vera* and results of Qiu et al. (2010) on *P. chinensis*. In this research, the researchers examined that cytoplasmic partition after meiosis is carried out synchronously by setting up channels from around microspore mother cell toward the center of cell and building up four microspores. These cells are oriented tetrahedrally. In this study, all tetrads were tetrahedral. Li et al. (2011) discovered that tetrad of *P. vera* is isobilateral, so there are both types of tetrad in varieties of *P. vera*. Qiu et al. (2010) informed that *P. chinensis* has both types of tetrahedral and isobilateral tetrads. In pre-meiotic phase, parts of microsporangia situated in one theca are secluded from each other via the septum. But in meiotic phase, middle indentation of each theca of anther turns into totally deep until it arrives in the central vascular bundle of anthers, so that the two microsporangia in a theca are totally secluded from each other. This stuff is also found in few plants. In most cases, the septum totally secludes two pollen sacs until the end of the maturation phase. At last, the two sacs are attached and let pollens out via shallow dehiscence pore. The post-meiotic phase starts with disintegration of callus wall and passing tetrad phase to microspore phase. At last, middle stratum and tapetum stratum vanish with distinction of fully-grown pollens. Fully-grown anther wall is equipped with the mechanical stratum with U-shaped motif along with the traces of the tapetum stratum and the middle stratum. Separation of the two middle walls of each sac with the central part of anther leads to dehiscence in length and depth. The pollens are detached across the deep cleavage locations.

Crops manage the establishment of pollen grains, eggs, and grains in reaction to environmental circumstance change. In extreme environmental circumstance, establishment of pollen grains will fail. In this research, the male gametophyte growth phase in pistachio plant was examined with standard methods and plant reaction to NaCl stress at this phase was observed. The findings yielded from this study is in a mutual accord with the result of prior studies on the preventative effect of salinity stress on male gametophyte growth in plants (Namuco and O'Toole 1986, Moss and Downey 1971, O'Toole and Moya 1981, Saini 1981, Sheoran 1996, Westgate and Boyer 1986). Namuco and O'Toole (1986) revealed that microspores are susceptible to salinity stress during the development phase. In some plants, including beans, canola, corn, and soybeans, stress circumstances lead to the mortality of plant cells in the mature gametophytes (Kokubun 2001, Moss and Downey 1971, Sage and Webster 1990, Young 2004). Consequently, the natural growth of gametophyte, embryos, and pollen in plants can be stopped or thwarted by environmental stresses, but the fiasco in the establishment phases relies on the phase where stress is applied. Male reproductive growth in plants is very susceptible to salinity stress and dehydration in PMC during meiosis. During this stage, the establishment of most of microspores or pollen grains is impeded by the water

deficiency and it leads to male sterility. A direct impact on reproductive tissues is seemingly not the cause of these injuries but an indirect impact of water deficiency in different organs like leaves is. The mechanism of this reaction may involve a long distance molecular warning in organs that are under pressure and influences fertility in reproductive tissues. A lot of studies notify the complicity of abscisic acid in this matter, but more clue is needed to verify the participation of this hormone in the induction of male sterility (Morgan 1980, McRae 1985).

The discontinuation of male gametophyte growth caused by stress circumstances is as a result of disruption of carbohydrates metabolism and distribution within the anther, and the stoppage of the sugar hydrolysis key enzyme, which is invertase. The glucose level can organize the gene expression of invertase. Salinity stress can cause the decrease in photosynthesis that will lead to the decrease in sugar distribution to the reproductive tissues and this could be a signal of the cause for metabolic changes, resulting in the fiasco of the male gametophyte establishment (Dorion et al. 1996, Saini et al. 1984)). The discontinuation of the male gametophyte establishment, that brings about the sterility of pollen grains, is the most typical among cereals. A sharp decline in crop production in many dicotyledonous plants is due to the drought led by the salinity stress during the growth of the stamen to the growth of anthers (Dubetz and Bole 1973; Salter and Goode 1967, Sato 1954, Turner 1993, Wells and Dubetz 1966, Westgate and Peterson 1993).

Saini et al. (1984) and Lalonde et al. (1997) have studied the anomalies in the development phase of anthers exposed to salinity stress and water shortage during meiosis. They pointed out that microspore stem cells apparently finalize the meiosis completely, but the development phase of more microspores is ended in the next steps. The most usual sign of the fiasco of development phase is the transference of microspores from their natural margin location. This can occur at any time among the young microspore step and the first mitosis of the pollen grain, and the period apparently depends on the cultivar. In some of the anthers, unusual vacuolation of tapetal layer after meiosis can be observed. Consequently, the incorrect function of tapetal may guide to incorrect orientation of microspores. Studies of Saini and Aspinall (1981) and Sheoran and Saini (1996) pointed that pollen grains with improper orientation and diluted cytoplasm have sheer antin or without it, but they own exine and compile no starch, that is the primary factor of productivity of the grass pollen grains. The anomalies in matching and dissociation of chromosomes during meiosis in pollen mother cells in the barley under the salinity and drought stress was originally observed by Skazkin and Zavadskaya (1957). These anomalies were identified as the cause of male sterility. Namuco and O'Toole (1986) also denounced that several chromosomal anomalies in rice are escalated under the stress of water shortage. In most cases, the results of this study explicated that the salinity circumstances in the flowering phase lower the quantity of pollen grains in anther. Moreover, anthers development phase is abnormal under salinity circumstance resulting some of anthers are

wrinkled, discolored, and small. The untimely cracking of the anther wall, the shrinkage of pollen grains, and the establishment of pollen with unusual shapes and properties verify that the salinity stress lessens the yield of Canola through impacting the male gametophyte development (Mahmoodzadeh and Bemani 2008). Learning separate environmental circumstances in the future and changes that take place during the growth phases of the pistachio and its reproductive organs is nominated. Saini et al. (1984) and Lalonde et al. (1997) examined the anomalies of anthers growth due to salinity stress and water deficit at the time of meiosis

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