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Research Paper

Study of Vegetative structure of Middle Eastern Raspberry

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Abstract

This research was conducted to investigate the vegetative structure of strawberry plant (*Fragaria vesca, L.*). In order to study vegetative parts of plant such as root, stem, leaf and petiole, manual cutting was performed and samples were placed in ethanol glycerin fixer. Samples were stained with Zaji Carmen and methylene blue. Shoot apical meristem of this plant was studied using microtome cutting. Shoot apical meristem was placed in FAA fixer and then it was stored in 70% Ethanol. After that, samples were embedded in paraffin mold and finally they were cut using a microtome. After deparaffinization, samples were stained with hematoxylin and eosin. The anatomical characteristics of the vegetative parts of strawberry plant are important results of this research. The vegetative structure of the root, stem, leaf, petiole and shoot apical meristem of this plant resembled other dicotyledon plants. The vegetative structure of this plant is similar to that of most flowering plants(angiosperms).

Keywords: Raspberry (*Fragaria vesca, L.*); Vegetative Structure; Shoot Apical Meristem

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1. Introduction

Fragaria vesca, L.(strawberry) is a herbaceous plant from Rosaceae family and it has a stem with two forms of sleeping (producer of adventitious roots) and upright. The latter form has a height of about 8 to 15 centimeter and it concludes to a flower at the end. Strawberry has trifoliate dentate leaves and bisexual arranged flowers. The set of calyx and corolla and androecium are joint together at the base and create some kind of shallow cup that in the center of it there is a bulge bearing separated carpels (1). Calyx consists of 5 green pieces and 5 calicules have covered it from the outside. In strawberry, calyx and calicules remain even after the ripening of the fruit. Corolla consists of 5 white petals and androecium consists of 30 stamens that arranged in 3 double circular rows (2). Strawberry inflorescence is a modified stem that ends at a primary bud. Pollens are matured before opening of anthers but anthers don't open until the flowers blossom. Vigour of pollens preserves for 2 to 3 days. Stigmas are ready to accept pollen for 8 to 10 days and fertilization occurs 24 to 48 hours after pollination (3). In strawberry, receptacle, which is covered by sepals, gradually becomes fleshy and contains abundant glucide storages and forms edible part of it. True fruits of plant are like tiny grains placed in the fleshy and edible part of strawberry and each of them has a hard cover that contains a seed (2). With the studies done, it was revealed that prior to this no detailed and comprehensive studies have been carried out in the field of anatomical and developmental properties of this plant. Due to the importance of this plant in various aspects, its anatomical and meristematic structure were studied using cellular and histology methods.

2. Materials and Methods

Vegetative parts of strawberry were collected in the spring from a strawberry farm located in Sufian Deh in Rasht and transferred to the laboratory. Shoot apical meristem of strawberry plant was

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placed in FAA fixer for 12 hours. Then, washing phase with running water was performed for 12 hours. After dehydration with increasing levels of alcohol and clearance with toluene, samples were placed in molten paraffin. Finally, after molding, cutting was done with the aid of a microtome so that the average thickness of the cuts were 8µm. After deparaffinization, coloring was done with hematoxylin and eosin. Thin cuts, with blade, were also prepared from other vegetative parts such as root, stem, leaf and petiole. For clarification, the samples were placed in 5% Javel water for ten minutes and washing was carried out with running water. In the next step samples were placed in 5% acetic acid for 5 minutes. After washing with running water, the samples were stained for 15 minutes with Zaji Carmen and after washing again with running water, the samples were placed in methylene blue for one minute. Samples were rinsed with distilled water and finally, the stained samples were placed on the slide and a drop of glycerin was placed on them and after placement of coverslip, they were prepared for microscopic observations. The samples were examined by a German Zeiss light microscope and pictures were taken with Canon digital camera.

3. Results

Root, stem, leaf and petiole of strawberry plant(*F. vesca*) were studied in terms of anatomical structure and the following results were obtained:

3.1. Investigation of root anatomical structure in transverse section

In strawberry plant, the roots quickly transform into initial secondary structure. In the transverse section of the root tissue, respectively from outside to inside, first there are rhizodermis cells, then the periderm tissue is located at the outer surface of the root, and then the parenchyma region of the skin is observed which consists of cells with cellulosic thin cell walls and is divided into two distinct regions. In the outer region, the cells are circular and separated by intercellular spaces. In the inner region, the cells approximately have a rectangular section and they are placed in radial rows and between them small intercellular spaces are found. Endodermis contains a row of cellular cylinder that marks the inner boundary of the root skin. In the radial walls of endodermal cells, casparian strips can be seen.

The vascular cylinder is located in the central part of the root. Endodermis in the root relative to the stem well determines the range of the vascular cylinder and the root skin. The vascular tissue is surrounded by a region of peripheral circle cells. Three poles of xylem or wood producers are observed. The secondary phloem tissue also continually surrounds the secondary xylem tissue. Root central core (medulla, stele or pith) is also observed and it is a set of parenchyma cells(Figure 1. A, B).

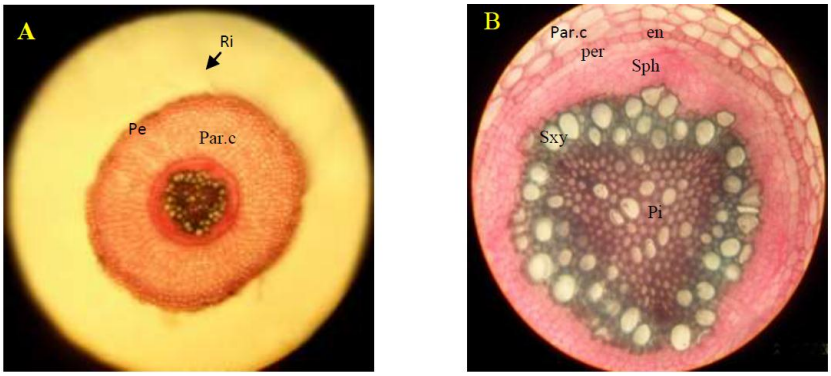

Figure 1. Cross section of initial step of root secondary structure in strawberry plant. A) (ob x4) and B) (ob x10.(Rhizodermis(Ri), Periderm(Pe), Cortex Parenchyma(Par.c), Endoderm(en), Peripheral Circle(per), Secondary Phloem(S.ph), Secondary Xylem(S.xy), Stem Pith(Pi)

3.2. Investigation of stem anatomical structure

In cross section of stem (Figure 2. A), the epidermal cells form the outermost layer that is made up of cubic cells. Immediately after that, the pink colored collenchyma tissue is located that are cells with thick walls and make up the strengthened tissue. Under the collenchyma, there are parenchymal cells that form the skin parenchyma and are arranged in several rows. Immediately after that, there is a green tissue called sclerenchyma fiber and after that a pinkish layer is observed, which is the phloem tissue. Then, the vascular cambium layer is located and under the cambium there is xylem tissue. The central part is composed of parenchymal cells which are almost seen in yellow. Within parenchyma tissue, crystalline structures in the shape of macle are observed. The vascular bundles in the stem are placed on a ring. Meanwhile, phloem is placed on both sides of xylem which is said to be so-called bicollateral. In the stem, protoxylem is placed toward inside(inward) and metaxylem is placed toward outside(outward),

which is said to be endarch primary xylem development (Figure 2. A, B).

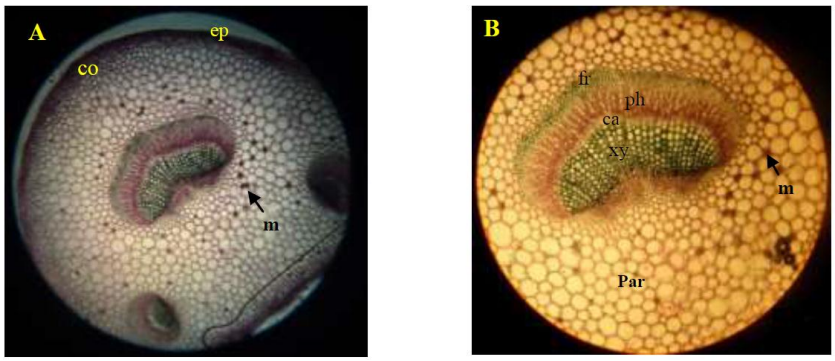

Figure 2. Cross section of stem in strawberry plant. A) (ob x4) and B) (ob x10). Epidermis(ep), Collenchyma(co), Cortex Parenchyma(par), Fiber(fr), Phloem(ph), Cambium(ca), Xylem(xy), Macle Crystalline(m).

3.3. Investigation of anatomical structure of leaf blade

In cross sections obtained from the leaf blade, from a histological point of view, there are three types of tissue (Figure 3. A, B): Epidermis consists of two upper and lower parts that surround mesophyll tissue. The epidermis surface is covered by cuticle. Mesophyll is a parenchyma tissue which is located between upper and lower epidermis. Two types of parenchyma is observed in mesophyll. Leaf parenchyma or mesophyll begins from upper surface and this section is composed of elongated cells(with small intercellular spaces) that are called palisade parenchyma cells. Mesophyll under the lower epidermis contains circular cells that are called spongy parenchyma cells which are placed irregularly whereas palisade cells are so regular. There are large spaces between spongy parenchyma cells, which makes loose their joints with each other.

Veins (leaf vascular bundles) bulge out on the lower surface of the leaf. In the veins section, vascular arrangements are observed in spiral form (Figure 3. C). In main vein, respectively from outside to inside the following tissues are observed: Epidermis tissue, under epidermis tissue there is collenchyma tissue which is supporting tissue and under it there is parenchyma tissue and under parenchyma tissue there is phloem tissue. In every vascular bundle, phloem is located around xylem and vascular bundle is bicollateral. A cap of fiber with cellulosic wall is placed on the phloem tissue bundles. There is a sheath of parenchyma cells around the vascular bundles (Figure 3. A).

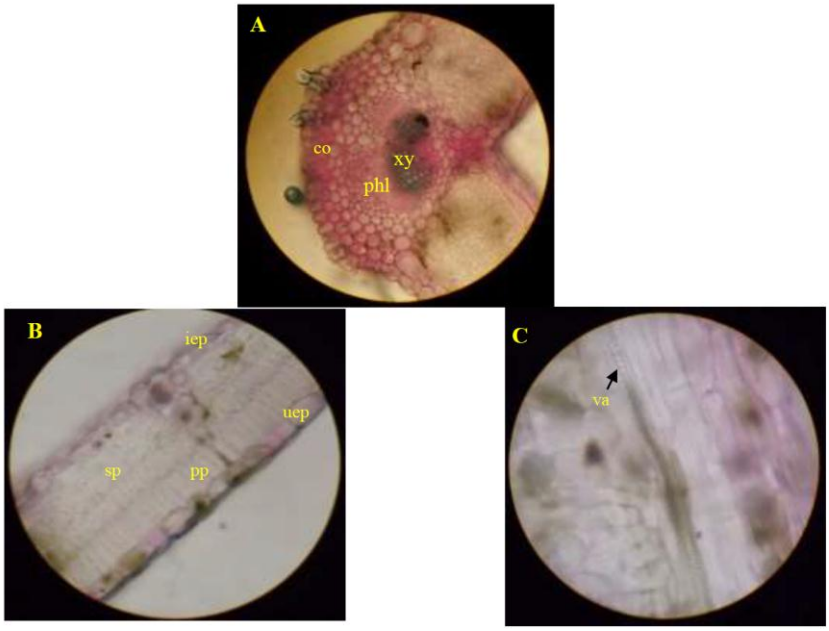

Figure 3. (A) and (B): Cross section of leaf blade (ob x40). (C): Cross section of leaf blade (ob x100). Upper Epidermis (uep), Lower Epidermis (iep), Collenchyma (co), Spongy Parenchyma (sp), Palisade Parenchyma(pp), Trichome (t), Phloem (phl), Xylem (xy), Spiral or Voluted Vessel (va)

3.4. Investigation of anatomical structure of leaf stalk

In cross section of leaf stalk from outside to inside the following tissues are observed (Figure 4. A): A dark layer which is epidermis tissue with cutinated surface. The most part of leaf stalk is occupied by pink colored multilayer tissue called collenchyma which is supporting tissue of leaf stalk. In the inner part of it, there is another pink colored tissue which is relatively lighter in comparison to collenchyma tissue and is parenchyma tissue which macle crystalline structure is also observed between parenchyma cells of leaf stalk like what is seen in the stem (Figure 4. A, B). Within each leaf stalk in the middle part of it, the vascular bundles are arranged in the form of an arc which is surrounded by a layer of parenchyma cells. Vascular bundles in leaf stalk are bicollateral like stem, i.e. phloem is located in both sides of xylem. The phloem is observed in pink color and under it there is yellow layer of cambium and after that the green layer of xylem is observed. On the epidermis surface, trichome cells are visible, which are of simple type (Figure 4. A).

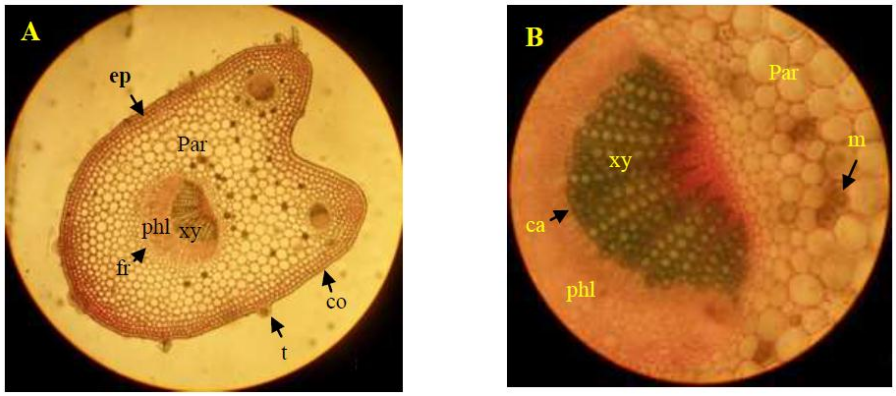

Figure 4. Cross section of leaf stalk in strawberry. A) (ob x4) and B) (ob x10). Epidermis(ep), Collenchyma(co), Trichome (t), Fiber(fr), Parenchyma(Par), Phloem(phl), Xylem(xy), Macle Crystalline(m)

3.5. Cytological structure of shoot apical meristem

In the longitudinal section of shoot apical meristem, the following parts are observed (Figure 5): Apical or terminal region (ma or meristem apex) including tunica in outer surface and corpus in the middle part, which is not very active in the germination step. Meristem peripheral zone (Iz) has two parts: A part with low activity which is closer to corpus and called resting or stagnant meristem. Another part is initiating ring with high activity which has many mitotic divisions and is the most active part of meristem because it has germinal centers of leaf and leaves will originate from it. This region has smaller cells, which is a confirmation of high activity and mitotic divisions. Stem is also originated from this region except for stem pith tissue which is originated from medulla part of vegetative meristem. This part is located in the innermost and central section of vegetative meristem. This part has lower mitotic division activity. Initiating ring like a cylinder surrounds the medulla meristem(mm). Medulla meristem is located in the central part of meristem and with its activity makes the stem pith.

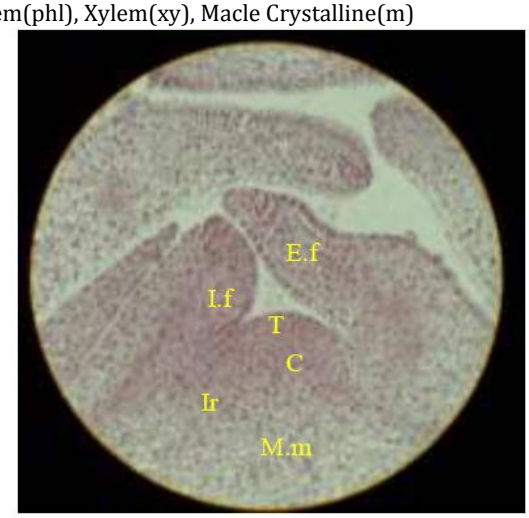

Figure 5. Longitudinal section of shoot apical meristem in strawberry. (ob x40). Leaf Primordium (E.f), Leaf initial (I.f), Tunica(T), Corpus(C), Medullary Meristem(mm), Initiating Ring(Ir)

Investigation of cross section of root structure showed that root rhizodermis quickly fell out and periderm replaced primary protecting tissue of epidermis. Other features of anatomical structure of the root are rapid formation of germinal layers of cortex and central cylinder. Phellogen with its activity creates the cork tissue toward the outside and cortex parenchyma toward the inside (4). Cambium layer, which is located between xylem

and phloem, toward the outside creates phloem and toward the inside creates xylem. A part of pith parenchyma penetrates between vascular bundles and creates the vascular ray. These properties are in accordance with common structure of root in dicotyledones (5) and reports of Majd et al. 2011(6). Finally, a layer of parenchyma cells regularly placed under endodermis and in alternation with its cells and creates the peripheral circle. Peripheral circle is the outer layer of central cylinder which is connected to the endodermis and its cells are in alternation with endodermal cells. The mentioned circle is generally made up of one cell layer (7). In strawberry root the peripheral circle is also made up of one cell layer. Root cortex is generally more extensive than stem cortex and has a greater role in the storage of materials. Parenchyma cells of root lack chlorophyll (8).

Strawberry has flowering stem, runner stem(stolon) and underground stem(rhizome). In cross section of runner stem, epidermis is located in the outermost layer. Epidermal cells are orderly and cubical, which from this perspective are similar to dicotyledones (4,9). Under epidermis there is cortex parenchyma or cortex which is identified by pink color and after that collenchyma tissue cells are seen, which are cells with thick walls and make the strengthened tissue. The next layer is cortex parenchyma cells in several rows with thin wall and approximately the same diameter. In the inner part of cortex parenchyma, there is a row of sclerenchyma tissue cells with green color. Because this tissue contains materials and compounds similar to xylem tissue, it absorbs the methylene blue stain. The function of the sclerenchyma tissue is providing strength and support to the plant(10).

After sclerenchyma we have vascular bundles of central cylinder(stele), which vascular bundles are arranged in bicollateral form. Bicollateral is said to a vascular bundle in which the phloem surrounds the xylem from both sides (5). In the stem, the protoxylem is found toward the inside and metaxylem toward the outside. That is why it is said that xylem development type in stem is endarch. In the stem, unlike the root, xylem pole is inward and this is one of the fundamental differences between root and stem. The innermost layer of cortex is endodermis which is composed of parenchyma cells that are tightly lied next to each other and like many other stems the endoderm is not well recognizable (9). Differentiation of phloem in the stem, like the root, is toward the center whereas development of xylem is in the direction away from center. The cambium structure, unlike the cambium in root, from the beginning is like an orderly ring. The results observed about strawberry stem are in accordance with the results of research by Bakhshi Khaniki et al. 2012(11).

Leaf blade in strawberry is egg-shaped with dark green color in the upper surface and light green in the lower surface and netted venation pattern (3). The epidermis is in both the upper and lower surfaces. Mesophyll is the parenchyma tissue inside the epidermis and usually from differentiation of its cells, the photosynthetic tissues containing chloroplast are formed. Two types of mesophyll parenchyma are observed. Palisade parenchyma tissue which with two cellular layers is located under the upper epidermis of leaf and does not exist in the lower surface of leaf, and it has cells with the same length or the length of cells become shorter toward the center of mesophyll, which confers a dorsal/ventral symmetry to the leaf. Palisade tissue is specialized for increasing efficiency of photosynthesis because it is situated in a site that obtains the maximum light. The presence of intercellular spaces in mesophyll is another factor that leads to increased efficiency of photosynthesis and possibility of gas exchange. Vascular tissue includes phloem bundles toward outside and xylem bundles toward inside.The properties observed for leaf blade of strawberry are similar to the structure of leaf blade of other dicotyledones such as Ivy(12). The active part of leaf consists of leaf blade and main vein. Main vein and leaf stalk have conductive or vascular system, which is the continuation of vascular system of stem in order to facilitate the passage of phloem sap, xylem sap and water. Vascular bundle is bicollateral and this was in accordance with reports by Bakhshi Khaniki and Moghsemi et al.(13) and Sherafatmand Attar et al.(14). The vascular tissue is enclosed by a sheath of parenchyma cells, that is called bundle sheath. Bundle sheath cells in dicotyledones are usually extended parallel to vein. Our observations are in accordance with statements of Fahn et al. (1989) about bundle sheath cells (8).

Epidermis is the outermost layer of leaf stalk which is seen in dark color. Its vascular bundles are seen in lunate shape or arched shape. The phloem is situated outside with pink color and under it there is the yellow layer of cambium. Under it there is green xylem tissue. A yellow layer enclosed the whole of vascular bundles, which is actually located outside of phloem tissue and called bundle sheath cells. Meanwhile, in leaf stalk, like the stem, the vascular bundles are bicollateral that phloem is situated around the xylem. The observed results are in accordance with the reports of Bakhshi Khaniki and Moghsemi et al.(13) and Sherafatmand Attar et al.(14).

Terminal bud in strawberry is dome shaped, which is in accordance with observations of Jafari et al. 2011(15) and Sirag et al. 2008(16). Shoot apical meristem is composed of several parts. The outermost layer called tunica layer, which surrounds inner cellular mass i.e. corpus. Tunica is

divided anticlinally and increases the surface while corpus is divided in all directions and increases the volume. These results are in accordance with observations of Jachson et al. 2008 (17). In the peripheral or margin of vegetative meristem, there is an initiative ring which is the most active part in terms of mitotic divisions and is the site of leaf and stem production except for the stem pith because the medullary meristem which is located in the middle of shoot apical meristem, creates the stem pith. Initiative rings have small and compact cells and have a great deal of ability to divide and proliferate. In the meristem of strawberry, tunica is situated in the apex and corpus is located in the lower surface of it and the initiative rings are around them. These observations are also in accordance with reports of Gerrath et al. 1993 (18) and Majd et al. 2011 (6).

4. Conclusions

Investigation of the results showed that anatomical structure of root, stem, leaf, leaf stalk and shoot apical meristem in strawberry plant is similar to that of other dicotyledones. Vascular bundles in stem, leaf and leaf stalk are bicollateral. In parenchyma tissue cells of stem and leaf stalk crystalline structures in the shape of macle are observed. Spiral vascular arrangements are also observed in leaf blade structure. Mesophyll includes a row of palisade parenchyma cells and 4 to 5 rows of spongy parenchyma cells which increases the possibility of gas exchange and efficiency of photosynthesis. In the middle vein only one vascular bundle were observed. Vascular bundles in the leaf stalk are placed separately on one circle. With the studies done in the literature and references, it was revealed that prior to this no detailed and comprehensive studies have been carried out in the field of anatomical and developmental properties of strawberry plant and the current research is done for the first time on this plant.

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