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Structural Analysis of Reproductive Development in Pistillate Flowers of *Laurus nobilis* L.

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Abstract

In *Laurus nobilis* L. (Lauraceae) the development of female flowers (pistillate), between floral meristem differentiation and fruit formation was inspected through histological sections and SEM techniques. The reproductive development of the female flower starts when the apical meristem converts into a floral meristem. Four tepals, four stamens and a carpel are developed from the floral meristem in turn. Filaments emerge however, anther development is arrested, and stamens become nonfunctional staminodes. The stigma is of the dry type. The solid style being short and thick consists of an epidermis, a cortex, a vascular bundle and a core of transmitting tissue composed of elongated cells. In the style a funnel-shaped zone extending from within the stigma to the stylar base is visible. The presence of high amounts of sugars and lipid substances within and around the vascular bundles are identified by histochemical techniques. The ovary contains an anatropous, bitegmic and crassinucellate ovule. Starch grains are present throughout the development of nucellar tissue. The chalazal region of nuclear endosperm forms a short haustorium. Endosperm does not exist in mature seed; the cotyledons are piled with considerably large starch grains. Idioblasts are observed in all stages of development.

Keywords: dioecious, idioblast, *Laurus nobilis* L., pistillate flower development, staminode

Introduction

Although unisexuality is very common in animals, hermaphroditism is the rule in angiosperms (Tanurdzic and Banks, 2004). In flowering plants approximately 10% of species are dioecious, producing flowers of one sex only on each plant (Dellaporta and Calderon-Urrea, 1993). Only stamens or carpels develop to maturity in unisexual flowers.

The start of flower bud development comprises the differentiation of vegetative meristem, its conversion into apical meristem and its transformation over again, into floral meristem in the upcoming stage (Bernier *et al*., 1993). The flower organs; sepal, petal, stamen and carpel develop from the floral meristem (Atsmon and Galun, 1960; Malepszy and Szczytt, 1991) but further development of stamens or pistils is selective, resulting in unisexual flowers (Haughn and Somerville, 1988; Rastogi and Sawhney, 1988).

The prevention of stamen or carpel development is a significant process that shows dissimilarity among species. The spectrum interval of unisexual flower development evolves from the differentiation of organ primordia until the formation of the sex organs which are completely developed but non-functional (Dellaporta and Calderon-Urrea, 1993). In dioecious Silene species, both the stamen and carpel primordia are present in both sexes, with the developmental arrest of the inappropriate sex occurring at early stages of floral development (Ye *et al*., 1991). The stage of arrest is later in maize, when the organ primordia are well defined but prior to their full maturation and meiosis (Dellaporta and Calderon-Urrea, 1993). The arrested development of the sex organs is not accidental; it is manipulated by the environmental conditions and genotype (Kinet *et al*., 1981).

One of the interesting issues in developmental biology is the sex differentiation and development in the flowers of monoecious and dioecious plants which carry unisexual flowers. Even though there is considerable knowledge on sexual differentiation in hermaphrodite plants, little information is present about sex differentiation in dioecious species and the prevention of opposite sex development.

Having gained a significant role in both economic and social life since the early ages, *L. nobilis* L. (sweet bay) is a dioecious species which has an aromatic smell and remains green not only in summer but also in winter. Sweet bay, which remains present mostly in Mediterranean countries, not only originated from Asia but is also a characteristic tree of the Mediterranean maquis.

Embryological features of Lauraceae were clearly reviewed by Kimoto and Tobe (2001). The present study of *L. nobilis* aims to contribute not only to filling the gap in embryological knowledge but also to understanding sexual differentiation and female flower development. We analysed the course of events starting from the initiation of the female flower up to fruit formation based on histological sections and scanning electron microscopy (SEM) techniques. In parallel, we examined the structure of stig-

ma and style of this species that is first presented in this family species.

Materials and methods

Plant Material

Flowers of *L. nobilis* at various stages of development were collected from the Göztepe Campus of Marmara University, from November of 2008 to June of 2009. Firstly, female flowers were morphologically analysed by stereomicroscope (Olympus 970931). The lengths of flower parts were mesaured and the samples were prepared for light and SEM analysis.

Preparation for microscopy

The material was fixed in acetic-alcohol (1:3, v/v) and then placed in a vacuum desiccator to facilitate the penetration of the fixative into the plant tissues. After embedding in paraffin, blocks were sectioned at 8-25 µm by Leica RM2235 rotation microtome and sections were stained with hematoxylin.

Histochemistry

For histochemical analysis, sections were stained with periodic acid-Schiff (PAS) (Feder-O'Brien, 1968) for insoluble polysaccharides, Coomassie brilliant blue (Fisher, 1968) for total proteins and Sudan Black B (Pearse, 1961) for lipids. The preparations were photographed with an Evolution LC color camera and an Olympus BH-2 microscope, and the images were analyzed with Image-Pro Express Version 6.0 scientific image processing and analysis software.

Periodic acid schiff's reagent (pas) treatment

The slides were immersed in a 1% (w/v) periodic acid for 20 min, rinsed in tap water for 10 min and stained in Schiff's reagent for 30 min in the dark. Then they were washed three times for 5 minutes each with 0.5% sodium metabisulfite and in tap water for 5 min. The slides were mounted in Entellan.

Coomassie brilliant blue treatment

The sections were stained in a 0.2% Coomassie brilliant blue (in a mixture of water, methanol and acetic acid (v:v:v, 87:10:3) for 10 min at 60^{0}C and transferred to water for 5 min. The slides were mounted in Entellan.

Sudan black B staining

The slides were immersed in Sudan Black B which was prepared immediately before use for 1 hour at 60^{0}C and rinsed in 70% ethanol for 2 times and one time with water. After drying at room temperature, the sildes were mounted in gelatin-glycerol.

Electron microscopy

For SEM analysis, the plant material was fixed in 2.5% glutaraldehyde in 50 mM cacodylate buffer, pH 7.0 (Platt *et al*., 1983) and then dehydrated with an increasing ethanol gradient: from 70% up to 100%. Then, the material for drying was kept in various percentages of ethanol-HMDS solution at room temperature (Topçuoğlu *et al*., 2009). Then, coated with 11 nm of gold by using an automated sputter coater and then examined with a SEM (JEOL JMS-59 10LV).

Results

The early developmental stages of female and male flowers are similar to each other. The visible differences occur after the sex organ primordia emerge.

Flower morphology

The flowering phase in female trees of *L. nobilis* happens between March and May. The potential flowering points are formed in October-November of the previous year. Mostly, flower buds come into existence at the growing points. Flower buds are rounder and larger, while leaf buds are thinner and more tapered (Fig. 1). Female flower buds exist in groups of five, at the tip of shoots (Fig. 1), covered by bud scales. Flowering doesn't start simultaneously at every flowering point. Inside the flower groups flowering develops from the center to outwards, thus the inner flowers mature more quickly than the outers. The length of flower buds, which is 1-2 mm at the early stage, reaches 4-6 mm at maturity.

The mature female flowers (3.9 mm) are smaller than male flowers. There are four tepals which are yellow and approximately 3 mm in length in a mature female flower. Inside the tepals there are four nonfunctional stamens (staminodes) (Fig. 1), whereas a male flower bears 8-10 fertile stamens.

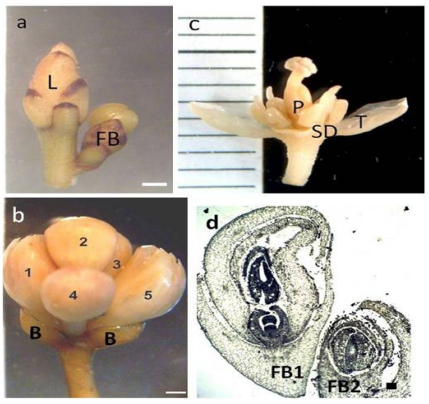

Fig. 1. a) Leaf and flower buds, Scale bar: 1 mm, b) 5 flower buds, Scale bar: 1 mm, c) A recently opened pistillate flower (a-c viewed by stereomicroscope), d) Longitudinal section of flower buds, Scale bar: 100 µm. B: Bracte, L: Leaf, FB: Flower bud, P: Pistil, SD: Staminode, T: Tepal

The nectarium, which is smaller in male flowers, has an approximate length of 1.3 mm and a width of 1.0 mm, and is located on both sides of the filament in the female (Fig. 2).

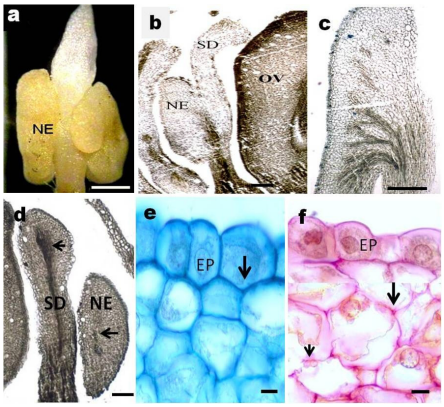

Fig. 2. a) A mature staminode and nectaries viewed by stereomicroscope, Scale bar: 1mm, b) Longitudinal section of staminode and nectarium stained by hematoxyline, Scale bar: 100 µm, c) Extensive networks of vascular bundles in nectarium, Scale bar: 100 µm, d) Longitudinal section of staminode, nectarium and vascular bundles stained by Sudan Black B, Scale bar: 100 µm, e), f) The nectariferous cells surrounded by secretory substances stained by Coomassie Brilliant Blue (e), stained by PAS (f) and starch grains, Scale bar: 10 µm. EP: Epidermis cell, NE: Nectarium, SD. Staminode, OV:ovary

Nectarium

In a pistillate flower, two nectaries are situated close to the base of a staminode (Fig. 2). Vascular bundles in the nectarium of pistillate flowers are present only below the nectariferous tissue (Fig. 2). The epidermis of the nectarium is covered by a cuticle (Fig. 2) and the nectariferous cells differ from those of the neighbouring tissues due to their bigger size and stronger positive reaction to Coomassie Brilliant Blue, PAS and Sudan Black B. They contain a small amount of starch grains at all of the development stages (Fig. 2). In an unopened flower, the whole nectarium is uniformly stained by PAS. In unopened and opened flowers, no changes in staining were distinguished. Subepidermal cells are surrounded by secretory substances giving a positive reaction to Coomassie Brilliant Blue and PAS, accumulating in the intercellular spaces (Fig. 2).

Flower development

While female flower development was examined starting from the apical meristem differentiation to fruit formation, the bud lengths are measured in order to identify the alterations that occur during the development, and being divided into eight stages, the development was analysed by stereomicroscobe, light microscobe and SEM (Tab. 1).

1. Initiation of floral meristem

Initially, the apical meristem differentiates from the vegetative meristem when the female flower starts to develop. Young leaves exist on both sides of the apical meristem, which consists of sequential cell layers (Fig. 3). The width of the floral meristem, which develops from the apical meristem, is more than the width of the apical meristem; however, the depth of the floral meristem is lesser (Fig. 3 and 4). The floral meristem cells in the female flower contain abounding cytoplasm, big nuclei and small and many vacuoles. The nuclei contain numerous nucleoli. The space between cells is considerably small among thin–walled meristem cells which form well-adjusted cell layers. Flower organ primordia, which are tepal, stamen and carpel in order, develop from the floral meristem (Fig. 3).

Tab 1. Morphological indications and respective lengths of floral buds in *Laurus nobilis* L. at various stages of development

Stage	Morphological indications	Bud Length (mm) n:25	Figure Numbers
1	Initiation of floral meristem	< 1.12	3b, 4b
2	Initiation of tepal primordium	1.21	3b, 4b
3	Initiation of stamen primordium	1.35	3c, 4d,5a-c
4	Initiation of carpel primordium	1.42	3d, 4f
5	Pistil development	1.64	6a-c
6	Megasporogenesis and Megagametogenesis	1.78	13 a-d
7	Endosperm and embryo development	2.37	13e
8	Seed and fruit formation	3.78	14a-c

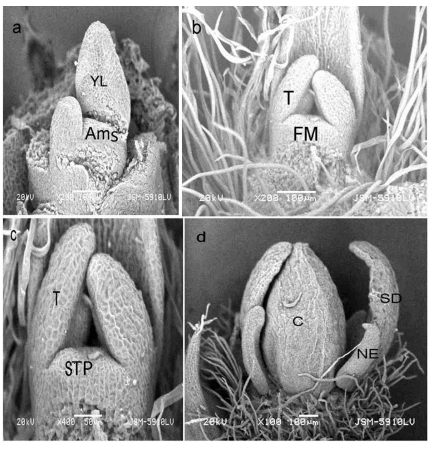

Fig. 3. Scanning electron micrographs of early development stages of the flower (outer flower whorls). a) Apical meristem, b) Floral meristem, c) Stamen iniation, d) Carpel and staminodes. YL: Young leaf, Ams: Apical meristem, FM: Floral meristem, T: Tepal, STP: Stamen primordium, SD: Staminode, NE: Nectarium, C: Carpel

2. Initiation of tepal primordium

Firstly, four light yellow tepals develop from the floral meristem in female and male flowers. Tepal primordia, which are located on the outermost first whorl, initiate from the floral meristem. The floral meristem enlarges and bulges are observed on the upper surface. Four tepal primordia develop consecutively and in whorl form (Fig. 4).

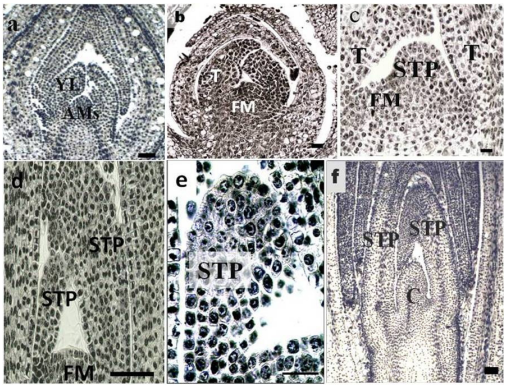

Fig. 4. Some early development stages in longitudinal sections of the flower. All bud scales have been removed in all. a) Apical meristem, Scale bar: 100 µm, b) Floral meristem, Scale bar: 100 µm, c)-f) Initiation of stamens and carpel, Scale bar: 100 µm (d, f), 10 µm (c, e). YL: Young leaf, AMs: Apical meristem, FM: Floral meristem, T: Tepal, STP: Stamen primordium, C: Carpel

3. Initiation of stamen primordium

In the beginning of the stamen primordium in the female flower, a group of cells divide and form a bulge from one part of the floral meristem and this bulge lengthens to give rise to the filament. A group of cells differentiate likewise from the other side of floral meristem and start forming the filament of the other stamen (Fig. 4), while the development of the filament continues (Fig. 4). Filaments keep growing longer in the stamens however the anthers do not differentiate (Fig. 5). It was observed that anther differentiation existed in none of the 200 experimental flowers. Stamen rudiments are visible on mature female flowers and form narrow rods in 2.2 mm in length. Staminodes hold two nectaries and they continue to appear throughout flower development. Staminodes degenerate at the end of the development (Fig. 5).

4. Initiation of carpel primordium

After the emergence of all stamen primordia out of the floral meristem is completed, the cells in the center of the floral meristem divide and differentiate as carpel primordium inside the stamen whorls. The carpel primordium arises as a small protuberance. When the floral meristem converts into the form of carpel, a curved appearance arises (Fig. 3, 4 and 6). The carpel primordium occupies most of the center of the flower bud.

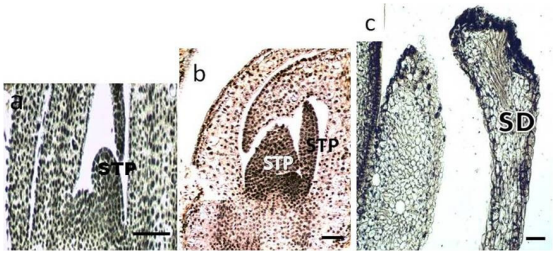

Fig. 5. a), b) Stamen primordium (STP), Scale bar: 100 µm, c) Staminode, Scale bar: 100 µm. STP: Stamen primordium, SD: Staminode, NE: Nectarium

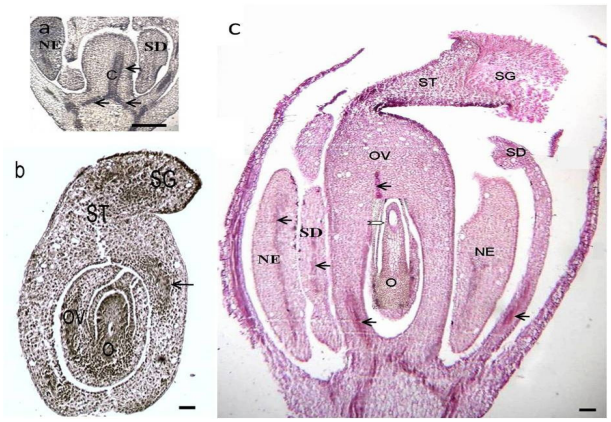

Fig. 6. Pistil development in *L. nobilis*. a), b) Pistil in an unopened flower bud, Scale bar: 100 µm, c) Detail of flower in longitudinal section stained with PAS: A complete pistil containing a stigma, a style and an ovary with one ovule and staminodes with nectarium, Scale bar: 100 µm. ST: Style, SG: Stigma, OV: Ovary, O: Ovule, NE: Nectarium, SD: Staminode, Vascular bundle C: Carpel

5. Pistil development

The carpel primordium starts to grow by active cell divisions and first the ovary then the cylindrical style emerges. The style continues to lengthen, and a wide stigma evolves out of its tip (Fig. 6). In upcoming stages, the ovule comes into existence as a bulge on the upper side of the ovary which differentiates from the base of the carpel (Fig. 6). A big nectarium and staminodes in pistillate flower are shown in Fig. 6. A pistil consists of an ovary with one ovule, a short style and a capitate stigma.

Stigma

In this species, the capitate stigma is papillate and of dry type. The stigma starts developing when the style is approximately 0.3 mm in length, and it surrounds the upper area of the stylar tissue (Fig. 7). In this stage, the megaspore mother cell appears in the ovule. The receptive surface of the stigma is composed of unicellular papillae (Fig. 7). The papilla in young pistils have abounding cytoplasm, small vacuoles and a centrally located nucleus. Its upper surface has a round shape (Fig. 8). While the stigma matures, vacuoles in the papillar cell enlarge, their cytoplasm decline, and the nuclei move to the basal. The papil-

lae enlarge lengthwise, and they gain a cylindrical outlook (Fig. 8). As development proceeds, the boundaries of the papillae become less noticeable and they attain a broken view. The nuclei become invisible in most of them (Fig. 8). The papilla lengths, being on average 38-45 µm when they first appear, reach an average level of 72-84 µm in mature stigma. A noticeable decrease in their diameters is observed from the beginning of the development to the end: the diameters of papillae, which are roughly 20 µm at the young stage, decrease to a level of 15 µm at the mature stage. A thick cuticle layer giving a positive reaction to Sudan Black B surrounds the papillae, and it is covered by a pellicle. This layer reacts positively to Coomassie Brilliant Blue, indicating the presence of proteins. With the maturation, the layer that surrounds the papillae starts thickening. At the young stage, the stigma forms a collapse (funnel shaped zone) which almost reaches the base of the style, inwards through the middle of the style. Long papillae are located, clearly on the surface of this breach. The width of the collapse increases with maturation of the style (Fig. 9).

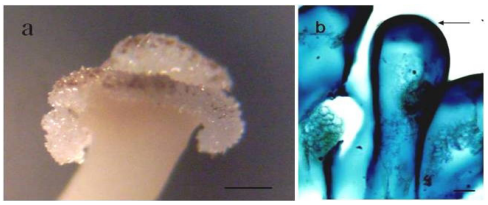

Fig. 7. a) Stereomicrograph of stigma in a mature flower, Scale bar: 250 µm, b) Stigma papilla stained with Coomassie Brilliant Blue, Scale bar:10 µm

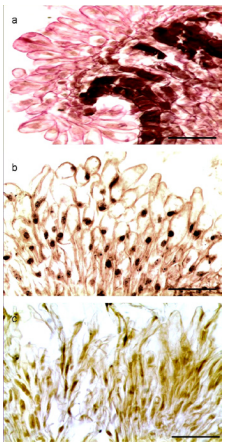

Fig. 8. a)-c) Longitudinal sections of young to old stigma papilla showing gradual changes, Scale bar: 100 µm

A longitudinal section of pistil stained with Coomassie Brilliant Blue and PAS shows that the papillar stigma is basipetally continuous with a closed style and is traversed by a core of transmitting tissue. The inner stigmatic cells are continuous with the stylar transmitting tissue, showing an overall funnel-shaped arrangement which is wider in the stigma and narrows basipetally in the style (Fig. 9).

Style

The style appears short and thick in the beginning of the development in *L. nobilis*. As the development makes progress, the style lengthens and its diameter increases as well (Fig. 9). The diameter of the style, which is roughly 0.4 mm in the early stages of the development, reaches a level of 0.7 mm in the final stages of the maturation and its length increases up to 1.3 mm. It remains bent towards one side of the ovary from the beginning of its development (Fig. 9).

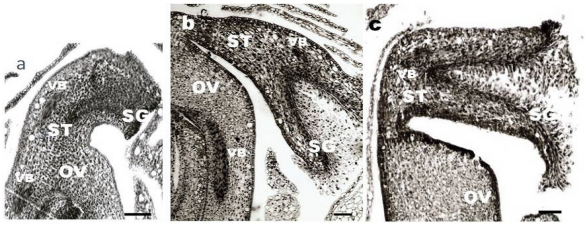

Fig. 9. a)-c) Longitudinal sections of young to old style with stigma showing gradual changes, Scale bar: 100 µm. OV: Ovary, ST: Style, SG: Stigma, VB: Vascular bundle

The style is solid and it is composed of an epidermis, a stylar cortex consisting of larger vacuolated cells organized in layers and a core of transmitting tissue (Fig. 10). One vascular bundle is visible on the side of the transmitting tissue and indicates a positive reaction to PAS (Fig. 6) and Coomassie Brilliant Blue (Fig. 10).

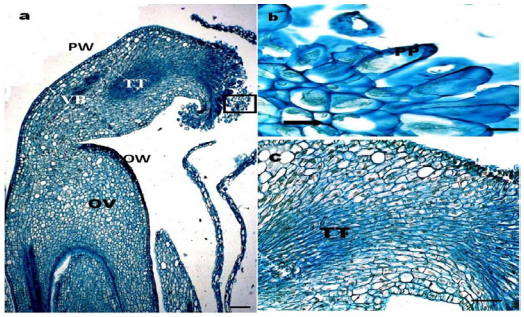

Fig. 10. a) Longitudinal section of a pistil stained with Coomassie Brilliant Blue, Scale bar: 200 µm, b) Details of stigma papillae and inner stigmatic cells. The pellicle and the intercellular spaces show a positive reaction, Scale bar: 10 µm, c) The transmitting tissue is also positive to this stain but shows a strong stained funnel shape, Scale bar: 50 µm. PP: Papillae cells, TT: Transmitting tissue, VB: Vascular bundle, PW: Pistil wall, OW: Ovary wall, OV: Ovary

The transmitting tissue is made-up of elongated, thick-walled cells which are aligned along the longitudinal axis of the pistil. While the cells which form the transmitting tissue are shorter and rounder at the young stage, they lengthen throughout development and become more cylindrical. At the mature stage, vacuolation increases in every cell of the style tissue, and the amount of cytoplasm

decreases. The decrement in the stainability of the cells is evident.

Cytochemical tests were applied to distinguish carbohydrates, proteins and lipids within the different tissues of the *L. nobilis* pistil. The cell walls of the pistil stain a pink color with PAS indicating non-soluble polysaccharides (Fig. 6), but staining is more intense in the stylar transmitting tissue and the contiguous non-papillate cells within the stigma (funnel shaped zone) and in the ovary epidermis (Fig. 6). The epidermal cell wall of the style (Fig. 6) and ovary also stain positively with PAS. The pistil vascular bundle shows a stronger reaction with this stain (Fig. 6). The cuticle of the ovary and style are stained with Sudan Black B. Cytochemical analysis indicated that intercellular substances of transmitting tissue show a strong stainability for the total protein (Fig. 10) and insoluble polysaccharides, as revealed by Coomassie Brilliant Blue and PAS reagent, respectively.

Ovary

The ovary is short-stemmed with one chamber with a hanging ovule. The fruit develops on the stem into deep black 2 cm long ovate berries.

Ovule

Ovule development in *L. nobilis* becomes visible subsequent to carpel enlargement. The ovule primordium emerges as a small protuberance of homogenous tissue and it bends in the course of development. The funiculus contains 1-2 vascular bundles, extending to the chalaza.

The ovule is bitegmic. The integuments appear close to the base of nucellus. Although the inner integuments differentiate earlier than the outer integument, the latter overgrows the former. In a fully mature ovule the outer integument is more massive and thicker (5-7 cell layers) as compared to the inner integument (2 cell layers). The micropyle is formed by only the inner integument (endostome). The integuments do not contain vascular bundles.

The ovule is crassinucellate; although, the parietal tissue above the megaspore mother cell is 9-11 cell layers thick, it consists of 16-18 cell layers at maturity. The nucellar tissue can be seen in a mature ovule. The nucellar cells throughout the development contain a large amount of starch grains giving a strong PAS positive reaction.

6. Megasporogenesis and megagametogenesis

Linear megaspore tetrad comes into existence as a result of meiotic division in the megaspore mother cell. The chalazal megaspore is functional. The 2–, 4–, and the 8–nucleated embryo sacs develop from the active megaspore through the sequential mitotic divisions. The mature embryo sac contains one egg cell and 2 synergid cells, 2 polar nuclei and 3 antipodal cells. Synergid cells are rich in cytoplasm, and they degenerate right after fertilization. From the applied PAS staining, it was evident that there was no obvious filliform apparatus.

Three prolonged spindle shaped antipodal cells are located in the chalazal part of the embryo sac. These are smaller compared to the other cells of the embryo sac, and being short-lived; they degenerete and vanish right after fertilization. The central cell contains large vacuoles and the secondary nucleus is round-ellipsoidal shaped. It was identified through the applied PAS reaction that starch grains were observed in the cytoplasm around the secondary nucleus.

7. Endosperm and embryo formation

In *L. nobilis*, the primary endosperm nucleus undergoes subsequent mitotic divisions and forms free nuclear endosperm which persists throughout. In a further stage of development the chalazal end of the endosperm forms a short haustorium reaching up to 0.65 mm in length and it remains free nuclear. In this species, the endosperm is consumed by the developing embryo in the early stage of development and after that the cotyledons support the nutrition of the embryo. The zygote is smaller than the egg cell as a result of the shrinking of the vacuole. Embryogenesis was not observed; however, a mature embryo was only seen in the seed of mature fruit.

8. Seed and fruit formation

The fruit remains as a one-seeded drupa. It matures between October and January, and it is oval-shaped, long-gripped and rigid. First it remains green, and then it turns into red, and ultimately, it becomes glossy black. The embryo is straight and has massive cotyledons in mature fruit. The fruit wall, the outermost epicarp layer of the pericarp, is a dark grey-black colored, rindless, skin-like and straight layer. It consists of thin-walled parenchyma. Mesocarp, the middle layer of the pericarp, is fleshy and thick and it is composed of loosely arranged parenchymatic tissues. The innermost part of the pericarp is the endocarp. The hardened endocarp is derived through the inner area of the ovary wall.

The characteristics of idioblasts

The idioblasts were encountered at every stage of development in the female flower. The idioblasts were evident in every flower part, from the early stage, in which the apical meristem appeared, until the late stages where the fruit tissue came into existence. Although morphological dissimilarity is not evident in idioblasts in various parts of the female flower, it is observed in the idioblasts in young and mature tissues. The idioblasts in young female flower tissues are smaller compared to those at later stages, and cells with big nuclei and vacuoles are located in them. A line of cylindrical shaped cell series are present in every flower tissue, lined up neatly around the idioblasts, in and after the stages where mature embryo sac cells exist.

Vascular bundles

In female flower an ovary, style, staminodes and nectarium contain vascular bundles (Fig. 6). An extensive network of veins feed the nectaria. Furthermore, a distinct vein supplies the staminode even though stamen development is arrested. Vascular bundles in the pistil tissues give the strongest reaction to Coomassie Brilliant Blue, PAS and Sudan Black B, indicating the presence of a high amount of proteins, insoluble polysaccarides and lipids, respectively. Vascular bundles in staminodes and nectarium show strong positive reaction to these stains as well.

Discussion

There are differences between leaf and flower buds which develop out of growing points in *L. nobilis*; leaf buds are pointed and long, and flower buds have small depth and broad expanse. Esau (1977) stated that the vegetation cone in some growing points inside the buds in the beginning of the morphological division period, is broader and swollen, while it is tight and flat in some of them.

At the very beginning of female flower development in *L. nobilis*, the apical meristem develops as a sharp bulge. As in dioecious *Silene latifoloia* (Grant *et al*., 1994), the apical meristem in *L. nobilis* is composed of uniform cell layers, and young leaves are present on both sides of the apical meristem. The transformation from apical meristem into floral meristem in *L. nobilis* is similar to that in the tomato. The apical meristem encounters many alterations during transformation into tomato: its volume increases, the apical apex comes into existence, the floral meristem appears following the flattening of the apex, and the flower organ primordia differentiate out of the floral meristem (Chandra-Sekhar and Sawhney, 1984; Grant *et al*., 1994). According to the examinations of Teeri *et al*. (2006), the flattening and broadening of the floral meristem is gained through the increase in the division ratio of the cells in the center, mostly.

Initially, the tepal primordia which exist on the outermost ring are formed out of the floral meristem in *L. nobilis*. Afterwards, the stamen primordia are constituted inside the tepals, and ultimately, the carpel primordium emerges in the center of the female flower. The flower ontogeny of this species is similar to that in Arabidopsis and Antirrhinum. According to this model, the development of flower organ primordia in plants is centripetal and sequential. Firstly, sepal primordia emerge in *Arabidopsis* and *Antirrhinum* flowers. Next, the petals, and immediately following that, the stamen primordia differentiate. Ultimately, the carpel primordium starts developing in the floral meristem center (Smyth *et al*., 1990; Sommer *et al*., 1990).

In some species, unisexual flowers might not illustrate any remainder of the atrophic sex. The development in the female flower of *L. nobilis* differs from this situation. For instance, female flowers in *Cannabis sativa* are able to pass directly from the periant development initiation to the start of carpel formation. These flowers do not carry a trace of the stamen primordium (Ram and Nath, 1964). Yet, we observed that filaments which contain 2 nectaries each differentiated in *L. nobilis*; however, anthers did not emerge. Thus; we identified that the staminodes that came into view were effective in the pollination of pistillate flowers.

A similar example to this development of the stamens in *L. nobilis* occurs in *Asparagus officinailis*. The critical phase in the sex determination in dioecious *A. officinalis* arises in the far future stages of flower development. The flower buds in female and male plants can not be distinguished phenotypically until the meiosis initiation (Lazarte and Palser, 1979; Bracale *et al*., 1991). In this stage, in order for the mature flowers to be unisexual, pollen development is arrested in female flowers, while the embryo sac formation is intercepted in male flowers. One of the breakdowns in stamen maturation in the female flower of *A. officinailis* is the development breakdowns in tapetum cells which emerge in the earlier stage (Lazarte and Palser, 1979). *L. nobilis* sex organ development differs from the development of the *Mercurialis* species, as well. Unisexual flowers in the *Mercurialis* species, which contains monoecious and dioecious species, are missing the sex organ primordia of the opposite sex from the floral meristem stage (Durand and Durand, 1991).

The carpel in *L. nobilis* starts differentiating with a dished appearence in the middle of the flower meristem. A similar development is encountered in the study by Verbeke (1992) on the *Catharanthus roseus*.

Almost all of the characteristics of female gametophyte development in 23 species of the Lauraceae family, which includes *L. nobilis*, were studied. Kimoto and Tobe (2001) demonstrated the embryological characteristics of the Lauraceae family in a reviewed study.

We compared the embryological characteristics of *L. nobilis* to this reviewed study, and we witnessed that a large part of its embryological characteristics were similar to those encountered in most of the Lauraceae members. The similar characteristics are as follows: Firstly, there is an ovary which contains a single anatropous, bitegmic and crassinucellate ovule showing apical placentation (Kimoto and Tobe, 2001). Also, meiosis in the megaspore mother cell results in a linear tetrad of megaspores and the functional megaspore on the chalazal side develops into an 8-nucleate Polygonum type embryo sac.

Antipodal cells are ephemeral. The nucellar tissue surrounding the mature embryo sac remains until the post–fertilization stages. The endosperm is nuclear. No obturator develops at the tip of the outer integument. A micropyle is formed by the inner integument alone. Finally, no endothelium is formed (Kimoto and Tobe, 2001).

We noticed that some characteristics which are seldom encountered in the Lauraceae family were formed in *L. nobilis*. These characteristics are as follows: The parietal cells are 9-12 layered in *L. nobilis*, but in Lauraceae a parietal

tissue above the megaspore mother cell is usually 2 to 6 cells thick. In Lauraceae family, usually a 2 to cell-layered nucellar cap is formed by the nucellar epidermis, but this does not exist in *L. nobilis*, as in Cassytha. The occurence of a haustorium is not common in this family; it was reported in *Cryptocarya chinensis* only. We observed a short chalazal endosperm haustorium which is free nuclear in *L. nobilis*, as well.

Idioblasts (fat cells) are encountered at every stage of development in the female flower in *L. nobilis*. Although their taxonomic values have grabbed the attention of botanicians for a long time (Baas and Gregory, 1985), very little information is available about the idioblasts (Platt and Thomson, 1992; Lersten and Curtis, 1993). It is known that their fat cells generate in various plant families such as Araceae, Aristolochiaceae, Calycanthaceae, Lauraceae, Magnoliaceae, Piperaceae and Aururaceae (Fahn, 1979; Baas and Gregory, 1985).

Idioblasts in *L. nobilis* were identified in every flower part, between the young phases where apical meristem tissues developed and the late stages in which the fruit tissue developed. Idioblasts are found intensely in the fruit wall in the Lauraceae family, especially in the mesocarp (Stefan *et al*., 2009).

There are few studies about the structure and development of the style and stigma in the Lauraceae family members which contain *L. nobilis*. In this species, the capitate stigma (Ghrabi, 2005) is papillate and of dry type (Watson and Dallwitz, 1996).

The pellicle layer surrounding the stigma papil in *L. nobilis* reacts positively with Coomassie Brilliant Blue, which is an indication that it contains protein. Extracellular proteins in the dry stigma are present in the form of an extracuticlar hydrated layer, the pellicle (Mattsson *et al*., 1974).

In *L. nobilis* the inner stigmatic cells are continuous with the stylar transmitting tissue showing an overall funnel-shaped arrangement which is wider in the stigma and narrows basipetally in the style. In this manner, the transmitting tissue in *L. nobilis* resembles that of the tomato as observed by Kadej *et al*. (1985), who showed it beginning as a broad column in the stigma and gradually narrowing in the style. It has been postulated that the architecture of the stigma and the style play a role in determining the pollen tubes that arrive at the ovule (Heslop-Harrison *et al*.,1985). A development similar to the stylar structure in *L. nobilis* is demonstrated in a study that was carried out in *Olea europaea* L. (Serrano *et al*., 2008).

Studies conducted on various angiosperm species have shown a correlation between flower abortion and starch content. A strong correlation has often been noted in woody plants between the successful development of fertile sexual organs and the amount of starch available in the flower at various stages of development (Rodrigo *et al*., 2000; Jean and Lapointe, 2001).

Histochemical analysis of sugars, proteins and lipids within and around vascular bundles in the pistil of *L. nobilis* suggests that they are involved in the transport of these substances, as observed in olives (Serrano *et al*.,2008). In this study (Serrano *et al*., 2008), the accumulation places of starch in staminate and hermaphrodite flowers are given in comparison, and ultimately it is pointed out that the male flower, in which pistil development is completed, differed from the starch dispersion in hermaphrodite flower, where the fertile pistil is developed.

Starch distribution in the entire pistil in *L. nobilis* was investigated, and although a strong PAS positive reaction was witnessed, it was observed that starch grains did not exist in the style or ovary. At every stage of development, the starch grains dispersed only in the nusellus in an intense manner, being present in a limited number in the nectarium, as well.

The results of this study provide important information about how the sex organ differentiates and develops while the other's development arrests. Moreover, our research contributes to the reproductive biology of this species that can be used in breeding programs.

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