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Biology of flowering and embryological structure of the iron tree in aderonic conditions

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Abstract

The article studies the development of the reproductive organs of iron tree, which belongs to the relict flora of the third period. It has been established that *Parrotia persica* has no serious obstacles that impede the normal process of seed formation. The flower group of this succulent species is almost devoid of cover. Moreover, it is mainly formed during the winter season, which limits the introduction of the plant into more severe climatic zones. İn the Absheron Peninsula, iron trees bloom and bear fruit only in years with mild winters, strong winds and the absence of night frosts. Embryological studies have established correspondence between the functions of the epidermis and endothecium, the presence in males of a clearly visible valve, in which the nuclei of the tapetal cells are located linearly in the radial direction, as well as a delay in the development of the female genital organs compared to the male system.

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Introduction

Parrotia persica (D.C.) C.A.-a unique botanical object with original biological properties. Maybe today it has become the center of attention of researchers. A number of aspects of the development process of this relict plant, which is one of the living relicts of the Tertiary period, including its embryology, have been practically not studied. *Parrotia persica*, considered one of the valuable woody plants, is naturally distributed only in the Hyrcan forests located in the Lankaran region of Azerbaijan, and in the local forests of the Gabala region. Due to the high specific gravity of this plant, it easily sinks in water, which is why *Parrotia persica* is popularly called the "iron tree". Observations show that shoots, flowers and leaves of iron tree can remain on the main plant for a long time.

It should be noted that the study of the intricacies of biology and embryology of the process of formation and flowering of the iron tree is still not at a satisfactory level. Therefore, studying *Parrotia persica* in the above aspects will further increase interest in the introduction and conservation of iron tree. Also of great importance in solving the problem is the study of the characteristics of the development of shoots in the iron tree, as well as the patterns of formation of the reproductive organs. It is on the basis of the results obtained in the course of research carried out in this direction that it is possible to conduct a comparative embryology of individual representatives of the family Hamamelidaceae. In other words, it is possible to find out what changes occurred in the elements of the reproductive structure during the adaptation of this ancient plant to modern environmental conditions along the path of morphological evolution.

Monotypic genus *Parrotia persica* (D.C.) C.A. Mey belongs to the Hamamelidaceae family. Representatives of this family were widespread in the third period. Conducted paleontological studies of this genus *Parrotia fagifolia* (Goepp.) Heer and *P. pristina* (Ettingsh.) Stur. shows that its species were once widespread. *Parrotiopsis jasquemontiana* Dens, found in the Himalayas (mountains of Kashmir). The species are very similar to members of the genus *Parrotia* in all their biological characteristics.

Representatives of the Hamamelidaceae family are more common in tropical and subtropical climate zones. These plants are not found in areas with adverse natural conditions. The first studies related to the study of the biological properties of iron tree in our republic were carried out by Safarov in 1960-1977 years. Thus, he conducted scientific research on the propagation of this plant by seeds, biology, ecology and phytogenology of its flowering, and its cultivation under cultural conditions (Safarov, 1972; 1977). In addition, although weakly, but also studied mycobiota of this tree (Muradov *et al.*, 2020). However, embryological studies, especially the development of reproductive organs of the iron tree are practically not carried out, although such studies (Janssens *et al.*, 2020, Rensing and Weijers, 2021) are important for understanding the general development of this tree. In this regard, the purpose of the presented work was to study the development of reproductive organs in the conditions of Azerbaijan.

Materials and methods

The material for morphological analysis and embryological research was taken from 30-35-year-old *Parrotia persica* trees growing in the Central Botanical Garden. To prepare stable preparations, the material was fixed in Carnoy's solution (6:3:1). Embryological material was prepared using the generally accepted cytological (Pausheva, 1974). To stain stable preparations, they are stained with both hemotoxin and Alcian blue solution according to Kederenghain. Pollen fertility was studied by staining with acetocarmine; viability was determined during germination in a 10-15% sugar solution by the drop method. Pollen morphology was studied using a fuchsin stain. Stabilized preparations were studied and photographed using MBI-6 and ZEISS Stemi 2000-C microscopes, Oxion euromex.

Results and discussion

Shoots

As a result of the studies, it was established that the shoots formed by iron tree are either vegetative or mixed in nature. At the end of autumn, morphologically identical shoots are brown and have short legs. Top shoots 4-5 mm, lateral shoots 2-3 mm. In autumn they become very hard and consist of 1-3 rudimentary leaves. Each of them produces two wild corns in a bud. Observations show that the iron tree does not have true shoot leaves, and their function is performed by pseudopods. To protect overwintering embryonic leaves and a certain part of the rhododendron from unfavorable environmental conditions, 10 or more dark brown delicate hairs are formed (Fig. 1, i1). Note the appearance of each hair under the microscope, the shape of an elongated mononuclear cell. The hairs are covered only by the leaves of vegetative shoots (Fig. 1, and 2). However, the outer surface of the flower bed and ovary is surrounded by cilia (Fig. 1, e; 3, a-d). Observations show that the active development of mixed and vegetative shoots of iron tree never begins simultaneously.

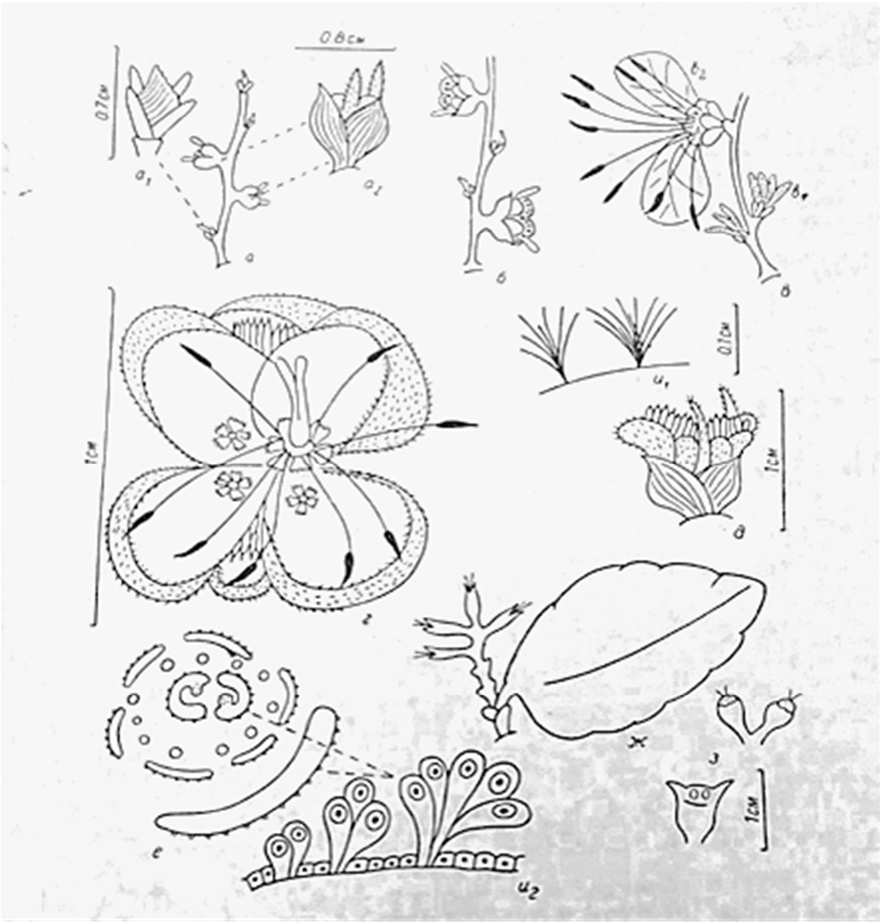

Fig. 1. Morphological development at different stages of flower and shoot

a- young branches (a1) and mixed shoots (a2) in December; b- January- February; v- vegetative shoots (b1) and mixed shoots (b2) (March); q- opened flowers; d- mixed shoots, two immature leaves in the axil of the flower (2 leaves per leaf, the tips of the shoots are deformed); e- cross section of a flower (petiole 1; to flower bed 6; stamens 11; petiole 2); j- young ovary; z- fruits; u1- bristles; u2- stages of hair development

Mixed shooting

These shoots form on the mother plant at the end of November. At this time, mixed shoots (Fig. 1a; 1a2) take on a rounded shape, and vegetative shoots in the spring are not quite similar to each other (Fig. 1a; 1a2). In January, the diameter of mixed shoots reaches 1 cm. Mixed shoots consists of one or two cotyledons, on each of which the heads of lateral flower buds are also visible. From which 5-7 flower-embryos are formed. During the flowering of iron ore, the flower crown formed from one flowerbed is bright green or dark brown on the inside (Fig. 1b; 1g; 1d), bare and drooping on the outside.

The number of corollas is equal to the number of flowers formed during flowering. Crowns are formed on peonies with a diameter of 7 mm in the flowerbed. Larger ones open in mid-January and 30-40 red stamens appear. At this time, the foundation of other elements of the flower is laid. However, their visual image has not yet been recorded. Therefore, before flowering in January, iron peonies exhibit bare and shiny stamens of a unique morphology. It should be noted that at this time the pollen is only in the uninucleate stage, and the basis of the seeds in the ovary has not yet been laid. Peonies overwinter in iron tree in a semi-open state.

Iron tree flowers grown in natural conditions are protected in this state by leaves remaining on the mother plant from the previous year. Introduced ironwood in Absheron conditions is affected by strong winds; flower development is delayed due to the fact that all the leaves remaining from the previous year fall off. Despite the fact that *Parrotia persica* is poorly protected from unfavorable winter conditions, its bare peonies are actively developing, and this adaptive feature can be considered an atavism. It should be noted that this plant grows well in warm weather under natural conditions. In the works of Safarov (1977) provides information about the existence of evergreen species of iron ore and other relict plants of the third period. Flower development in peonies begins almost synchronously. The number of flowers arranged cyclically around the flower bed varies from 5-7. The number of stamens in a flowerbed is unstable, fluctuating between 9-11 and they are located opposite each other.

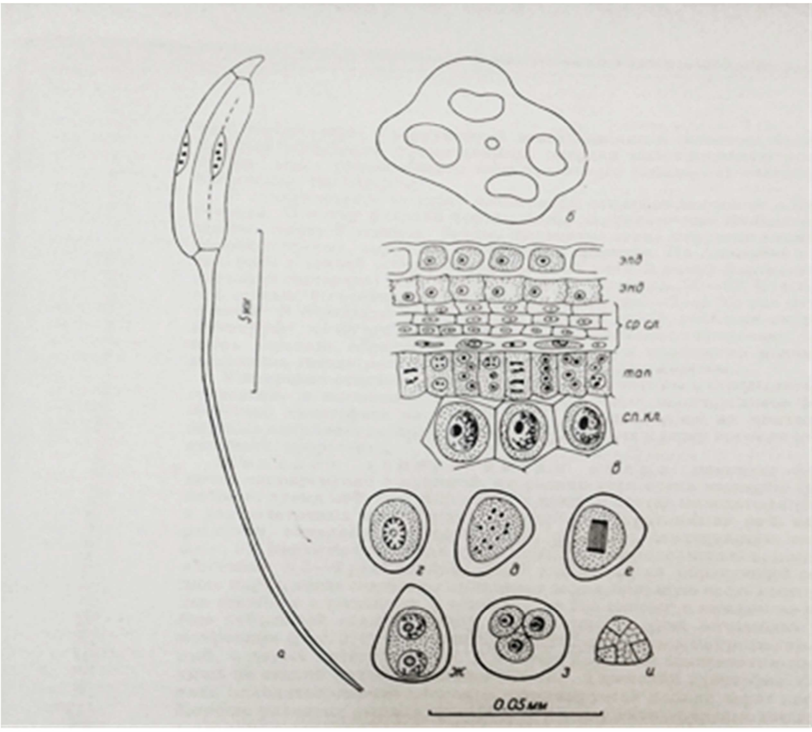

Fig. 2. Formation of the male reproductive structure

a- initial detection of the stamen in the anther, b- cross-section of the anther, v- transversely cut anthers; v- anther wall in the meiositis stage (December), q- diakinesis, d- metaphase, e- telophase I, j- prophase II, z- microspore in a tetrad, u- seed germinating from the anther (end of February), stained with magenta; en, kl- cellular sporogens, or. fold.- middle layer; find- tapetum, end.- endothecium, epi.- epidermis.

Formation of pollen sacs and anthers (Fig. 2a-2u). The generation and differentiation of the androecium begins in late November - early December. Young pollen sacs, reaching a height of 1.5 mm in mid-December, soon begin to turn red-pink and end with meiosis. During this period, the wall of the pollen sac becomes 6-7-layered, and the cell wall has a thickened epidermis.

The cells of the subepidermal layer, i.e., endothecin, unlike other angiosperms, lack thickened fibrosis. These cells increase in size at different stages of pollen sac formation and often undergo changes. In this case, these cells undergo lysis along with the middle layer and tapetum. Characteristics of the morphological features of the endothecium and epidermal cells show that the specific functions of the endothecium are performed by the epidermis. The middle layer is 4, and the innermost layer is flattened. The secretory tapetum is homogeneous. The cells taken here had 1–4 nuclei and were highly elongated in the tangential direction. In tapetal cells, the location of the nuclei on the internal longitudinal axis is of great interest. The width of the tapetal layer is equal to the width of 3-4 middle layers. In microsporocytes, meiosis usually occurs synchronously. At this time, the number of haploid chromosomes is n=12, and microspores are located tetrahedrally. Thus, although the characteristics mentioned above, including the multi-layered pollen sac, the thickness of the cell walls of the epidermis, the strong connection between the upper and lower parts of the beak-shaped stamen, seem primitive from an evolutionary point of view, they are extremely important in the protection of ironwood pollen, which is a living relic this third period, not only from heat, but also from cold.

In early January, young microspores emerge from the shell in the form of tetrads. At the end of February, two-celled reticulated seeds covered with reticulate exine are formed. Ripe reticulated iron ore seeds are located in the meridional direction with three grooves and have a round shape with a diameter of 46 microns.

On Absheron, in warm autumn and windless winter, the fertility of ironwood pollen is 90-92%; in a year of hot autumn and cold winter (-2; -4 C), the birth rate drops to 40% or less. In some cold years, ironweed only vegetates and does not bloom (this happened in 2017). The dust particles cracked longitudinally. First, pollen nests bloom in the middle part along the entire length of the pollen grains.

The ironwood matures and spreads faster than the embryo, which precedes the female gametophyte cells by 15-20 days. Pollen, spilled from pollen grains, sits tightly at the mouth of the pistil and at this time begins to grow.

The female generative sphere is formed simultaneously with the male one, and this happens very slowly (Fig. 3a-3o). During meiosis in December, each of the young flowers reveals a pollen sac and two cotyledons. It should be noted that throughout the winter they grow slowly, the lower parts unite and two nest ovaries are formed. The upper free part of each embryonic ovule ecomes a column and is 3-4 times longer than the entire lower part of the formed ovary. In mid-March, during the ripening of pollen grains, two styles are formed at the mouth of the pistil for most of the genesis. The seeds of the embryo in a mature state have the shape of a crassula and have two directions. During flowering, the seeds of the embryo are at the archesporial cell stage. But female gametes are ready to receive elements capable of reproduction, which begin to form after 2-3 weeks. The female's well-developed mouth holds the ripe pollen grain until the embryo sac is ripe to receive it. With weak growth of the pollen tube on the surface of the pistil, the only archesporial cell inside the style is located 10-12 times lower than the nutcellus cell, from which the megaspore is formed, which undergoes meiosis. As a result, after several mitotic divisions, a seven-celled, eight-nucleated female gametophyte of the Polugonum type is formed. The polar core remains ready for a long time, only merging with each other near breeding. Synergid cells have a stalk-like shape, the number of antipodes is 3. The cavity of the embryo sac is not very large.

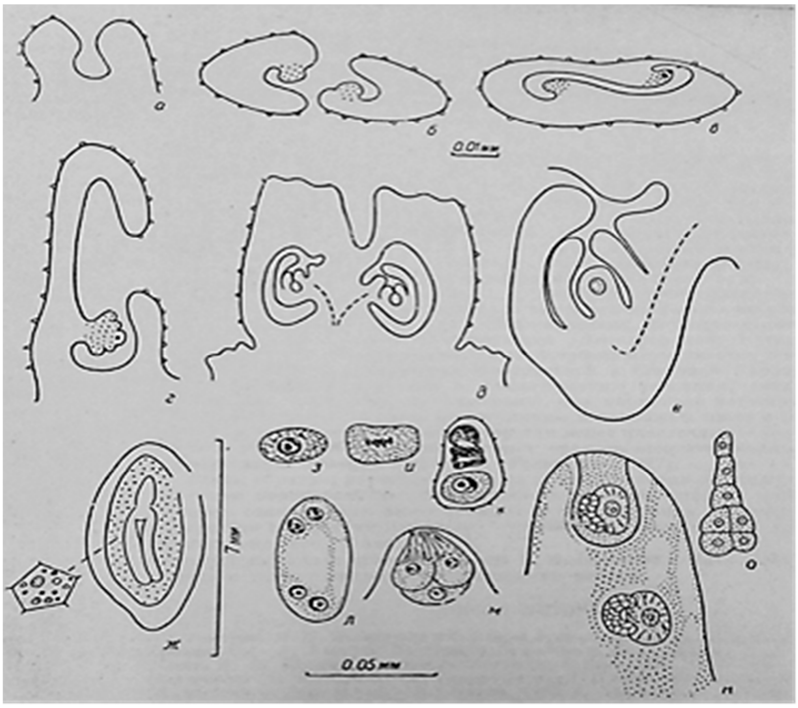

Fig. 3. Formation of the female reproductive structure
a- young fruit leaves (longitudinal section, December), b- development of the ovule (cross section, March), c- young ovary, q- ovary before flowering, c - longitudinal section of the ovary (April), d - mature ovary, j - mature seed, h, i- mother cell of mechaspore (end of March), j- mechaspore in tetrad, l- embryo sac emirio (beginning of April), m- nuclear apparatus, n- double fertilization (beginning of May), o- proembryo (4-5 weeks after fertilization, May).

Each ovary produces two anatropes, sessile seed shoots and integument. The period between pollination and reproduction takes 15-20 days. The division of the zygote begins after the endosperm has 8-12 nuclei. In parallel with this, the process of cell formation occurs. The first two-week-old embryo develops according to the Soland type, the embryo becomes filamentous Kaul (1969). It should be noted that embryo differentiation lasts 3-4 months. At the end of August, the cavity of the germinating seed is filled with an embryo and consists of a rhizome, a hypocotyl, two strong seeds and a shoot. The iron tree embryo lacks chlorophyll. According to Masieva (1972), planting fresh seeds in the fall gives 80% germination in the spring. It has been established that in the iron tree, when the seeds begin to increase in size, they do not contain either endosperm or embryo. Therefore, the embryo sac, which is not prone to reproduction, undergoes degeneration and persists for four weeks after pollination.

In such embryo sacs, antipodal cells are always well developed and resemble an egg. At the stage of formation of young fruits in İron tree, when the peduncles fall off, the distance between the nodes increases, the head of the peduncles increases 2-3 times and is retracted into a soft, erect stem. Endress (1993) confirms in his studies such a transformation of the cockroach during ontogenesis. As can be seen, in the third period the flowers are collected on a soft stem in favorable environmental conditions for flowering plants of the Hamamelidaceae family. However, as a result of the influence of cold climatic conditions, the distance between the nodes is reduced and peonies are formed according to the capitate type. The ironwood fruit consists of two seed pods.

Speaking about the history of iron trees, back in the 40s of the 20th century, Grossheim (1940) classified this plant as a relic with a wide ecological amplitude and Safarov (1977) confirms this idea in his work. At present, this plant grows in Lankaran, Gabala, Zagatala, Absheron, etc. It is also successfully grown in regions of Azerbaijan with different ecological conditions (Mamedov *et al.*, 2016). It should be noted that iron tree has a normal growing season, but its flowering and seed production are irregular. The generative organs of İron tree are characterized by low elasticity.

Conclusion

The study of shoot development, flowering biology and embryology shows that no deviations were allowed during these processes. The study of a number of unique features and structural elements in the development of İron tree is one of the evidence proving the relict nature of this plant.

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