Structural and functional features of the rosaceae determining passive immunity to fungal infections

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Abstract. With the help of modern microscopic methods of screening of the surface tissues of leaves and fruits of plants of the Apple subfamily (Maloideae): Apple (MalusMill.), pear (PyrusL.), quince (CydoniaMill.), medlar (MespilusL.) to model an object, and made before, the first attempts to explain the dependence of the susceptibility with the mycosis from the microstructural features. From the phytopathological studies and literature analyzed the species composition of fungi, causing loss of leaves and fruit of Apple trees in southern Russia. Outdoor, making pathogens with different parasitism are common pathogens, more reliable, more presented in the Mespilusgermanica. Higher, compared to the Apple and pear, a complex resistance to fungal diseases is found in the quince and medlar. Resistance to the first stage of the pathological process associated with such a device has, calmiramuniversity fruits and stomata in the abaxial epidermis of the leaves. Ulichnye cracks the leaves of the medlar - narrow, with raised growths on the surface of the fruit - now the structure of the cuticular layer.

1 Introduction

Apple (maloideae, or pomoideae) – one of the subfamily of pink (rosaceae), including the oldest cultivated representatives of angiosperms, widely distributed in many ecological and geographical areas.it includes such well-known fruit plants as pear (pyrusl.), apple tree (malusmill.), quince (cydoniamill.), medlar (mespilusL.) And other. Their main feature is the fruit overgrown with modified hypanthium-prefabricated leaflets or bony modified carpels, called in a broad sense apples [1-2]. A large (juicy) part of the apple is formed due to the succulence of hypanthium tissues [3-7].the typical apple is typical of apple, pear and quince, and the bony apple is typical of the german medlar (mespilusgermanica).

On practical importance in world production, including in russia, among fruit crops the leading place is taken by an apple tree, the second-a pear. The huge consumer value of the fruits of these crops, which are widely used in food, medical, perfume and other industries,
is well known. Along with vitamin-bearing qualities, the special value of apple and pear fruits is due to good antioxidant properties. Despite the huge number of varieties currently available, there is a need to improve the assortment, because in the process of long-term breeding, many valuable features that their wild relatives possess are lost.

Among the promising crops is quince ordinary (cydoniaoblongamill.). It is quite plastic to growing conditions, especially soil, can successfully grow on different types of soils (alluvial, chernozem, etc.). In addition, quince has a high resistance to various stressors, it is light-loving, heat-resistant, salt-resistant, quick-fruiting, and quite productive culture. C. Oblonga fruits are characterized by high technological qualities, rich in vitamins, organic acids, trace elements, pectin and other substances. Some researchers note that quince is less damaged by pests and diseases than other fruit crops from the subfamily maloideae [3-9]. Despite the fact that quince in its characteristics is a promising fruit crop, especially for the southern regions of Russia, to date it is not given due attention, and industrial plantations do not exist yet.

The group of promising fruit plants of apple (maloideae) includes the german medlar (mespilusgermanica), which is represented by two ecotypes: xerophilic, confined to open habitats, and mesophilic, forest [4]. In Russia, it has a wide distribution in the mountain ecosystems of the southern regions, especially in the north caucasus - from the lower zone to the upper limits of woody vegetation (370-2000 m above sea level), as well as in the crimea and adjacent territories, however, industrial plantations do not exist. The value of medlar has been known since ancient times. As a vitamin and medicinal plant, it has entered the culture of many countries of asia minor and asia minor, the eastern mediterranean and western europe. In vegetative and reproductive organs of m. Germanica, many biologically active substances are found, in particular, triterpenoids, tannins, phenol-carboxylic acids, flavonoids, catechins, organic acids and their derivatives, aliphatic aldehydes, higher fatty acids, a significant amount of fatty oil in seeds [7-8].

The development of maloideae diverse habitats and increased adaptability of representatives are based primarily on the development of structural and physiological and biochemical characteristics, which also play an important role in resistance to phytopathogenic organisms. for research in this regard, representatives of the above four genera were taken as model objects.

The anatomical and morphological features of passive immunity, or horizontal stability, include the specifics of the deposition of wax deposits and the thickness of the cuticle of the integumentary tissues, the structure of the stomata and a number of other features [9-10].

The material accumulated to date for some representatives of apple trees on the structure of surface tissues (epidermis and hypodermis) of vegetative and reproductive organs, as well as on their mycobiota, is fragmentary [11]. There is insufficient information about the multifunctionality of epidermal tissue, the specificity of the micro-relief of the surface of the epidermis of leaves (the formation of cuticular folds, micro-ligaments in the stomata and trichomes, peristomatic rings, cork tissue and lentils on fruits). Their functional significance is not entirely clear to date. In the literature there are disagreements and assumptions about the mechanism of opening and closing stomata, as well as the interaction of the latter with the surface structures of the leaf [10]. The role of the cuticle ultrastructure of leaves and fruits in passive immunity for many pathogens also remains controversial, and no direct dependence on the type of structure and overall thickness has been revealed.

The chemical factors of passive immunity include, firstly, the content or absence in plants of substances necessary for the life of the pathogen, and secondly, the presence of substances that depressingly act on it (phytoncides, or phytoanticipins). The leading role in the formation of the latter belongs to the synthesis and dynamics of various compounds of phenolic nature (from relatively simple phenols to complex polyphenolic composites), toxic to many phytopathogenic fungi [11]. According to modern ideas about the importance of
the features of the structure of the surface and subcellular structures of plants in protection from phytopathogens, a large role belongs to the synthesis and dynamics of phytohoromones [5]. At the same time, the distribution of lipophilic components, the specifics of the formation and localization of polyphenolic and terpenoid substances, which also play an important protective role against the attack of fungal pathogens in surface tissues, are insufficiently studied. It is shown that the phytoncidal effect of plants on parasites specialized to them, as a rule, is weakly expressed. In most cases, it spreads to microorganisms that do not affect this plant. Phytoncidal activity varies, it varies depending on the variety and age of the plant, as well as the time of day, phase of development, weather conditions and other factors.

Thus, despite the availability of some information on the biology and disease infestation of the most valuable fruit representatives of the subfamily of apple trees, no attempts have been made to comprehensively study their mechanisms of resistance.

In connection with the above, the purpose of this work was to conduct a comprehensive search study of micromorphological, anatomical and histochemical, as well as ultrastructural and physiological-biochemical characteristics of plants of 4 genera of the subfamily of apple, and evaluation of these indicators as markers of passive immunity to phytopathogenic fungi.

2 Methods

The objects of research (botanical and phytopathological) were the leaves and fruits of plants of different varieties of apple (malusdomestica), pear (pyruscommunis), collected from experimental sites of the north caucasus research institute of mountain and foothill gardening, as well as quince (cydoniaoblongam). From the planting of private plots, and wild medlar germanic (mespilusgermanica), growing in high-altitude zone in the north caucasus. Leaves and fruits for research were selected from the middle part of the crown of model trees.

To detect plant diseases and identify their pathogens, we used chamber methods, microbiological with the isolation of pathogens on a universal artificial nutrient medium (potato-glucose agar), followed by microscopy of infectious structures.

Microscopic (light) and histochemical studies of fruits were carried out on the microscope axioimager d1 (carlzeiss, germany) in transmitted light in the ifr ras named after k. A. Timiryazev. Sections with a thickness of 50 microns were made using a microtome with a vibrating blade (thermoscientific, microm hm 650v). To detect condensed polyphenols, the sections were treated with potassium bichromate (k2cr2o7) [20]. The micrographs were obtained using the axiocammrc camera (carlzeiss, germany); the images were processed using the zen lite 2012 program (carlzeiss, germany).

The microstructure of the surface of leaves and fruits was studied using a scanning electron microscope (sem) in a high vacuum mode and by spraying gold on the basis of n. V. Tsitsin gbs with the participation of a. S. Ryabchenko, to whom the authors are grateful for their assistance.

Autofluorescence of the surface structures of leaves and fruits was studied using a confocal microscope "olympusvf1000d" when excited by light 405, 473, 560 nm. Preparation of the material for electron microscopic studies of the content of polyphenols in the integumentary tissues (tem) was carried out according to the previously modified method [12-166]. The material was fixed with glutaraldehyde (on 0.1 m phosphate buffer with ph=7.2) and 1 % osmium tetroxide solution. The samples were then dehydrated in a series of alcohols and acetones of increasing concentration and poured into epon-812. Ultrathin sections were made on ultramicrotome lkb-iii-8801a. The sections were contrasted with 2 % aqueous solution of uranyl acetate (37°c) and lead citrate according to reynolds.
3 Results and Discussion

Based on the results of phytopathological studies in various plantings of the southern region of Russia, as well as the analysis of literary data [1, 9, 12,], a summary table on the main fungal diseases of leaves and fruits of plants of the subfamily Maloideae (Table 1).

Table 1. Fungal diseases of leaves and fruits and their pathogens in representatives of the subfamily Apple (Maloideae)

| Apple Tree (Malus Mill.) | Pear (Pirus L.) | Quince (Cydonia Mill.) | Medlar (Mespilus L.) |
|-------------------------|----------------|-----------------------|---------------------|
| 1(a1), 2(b), 4(d), 5(e), 8(g1,2,3), 10(h), 12(j1), 14(l1), 15(m1), 16(n), 21(o1), 23(p), 24(s) | 1(a2), 2(b), 4(d), 5(e), 6(f1), 8(g3), 10(h2), 11(i1), 12(j), 14(l2), 15(m1), 16(n), 21(o1), 22(o6), 23(p), 24(s) | 3(c), 4(d), 6(f1), 8(g1), 11(i2), 14(l1), 15(m1), 16(n), 17(o1), 22(o6), 23(p), 24(s) | 4(d), 7(f2), 9(g5), 10(h1), 11(i1), 12(j2), 13(k1), 14(l1), 15(m2), 16(n), 18(o2), 19(o1), 20(oa), 22(o6), 23(p), 24(s) |

1-Scab (a1-Fusicladium dendriticum (Wallr.) Fuck., C the columnar stage Venturia inaequalis (Cooke) Wint.; a2-F. Pirinum Fuck., C the columnar stage V. pirina Aderh.); 2-olive Mildew (b-Cladosporium herbarum). Alternaria tenius Nees.; 3-Anthracnose (c – Cylindrosporum cydoniae (Mont.) Schoschiaschwili (=GloeosporiumcydoniaeMont.)); 4-Anthracnose, bittersweet (d-Colletotrichum fructigenum (Berk.) Vassil. (=Gloeosporium fructigenum Berk). glomerella cingulata (Ston.) Sp. Et Schr.); 5-Flycatcher (e-Leptothyrium pomiSacc.); 6- Barbastesti, burapachaisri (f1-Entomosporium maculatum Lév. f. maculate Kleb. the columnar stage of fabraea maculata (Lév.) Atk.); 7-Dark brown spotting (f2-E. mespili Sacc.); 8 – Phyllosticta, burapachaisri (g1 – Phyllosticta mali Pr.et Del.; g2-P. Briardi Sacc.; g3-P. Pirina Sacc.; g4-P. Cydoniae var.cydoniicola (Allesch.) Cif.); 9-Phyllostictiosis, brown spotting (g5-P. Mespili Sacc.); 10-Spotting (h1-Hendersonia mali Trum.; h2-H. Pirica Sacc.; h3-H. Mespili West.); 11-Septoria, white spot (i1-Septoria pirica Desm.; i2-S. Cydoniicola Trum.; i3-S. Mespili Sacc.); 12-Ascochytosis (j1-Ascochyta pirica Sacc.; j2-A. Mespili West.); 13-brownish Spot (k-Asteroma mespili Rob. et Desm.); 14-Rust (l1-Gymnosporangium juniperinum (L.) Mart.=G. Tremeloides Hartig.; 12-G. sabinae (Dicks) Wint.; 13-G. confusumPlowr.); 15-powdery Grass (m1-Podosphaera leucotrichaSalm.; m2-P. oxyacanthae DB.); 16 – Sarahsilverman (n – Botrytis cinerea Pers.); 17-Moniloz, spotted leaves (o1-Monilia cydoniae Schell.); 18-Light brown spotting (o2-M. Linhartiana Sacc.); 19-yellow Spot (o3-M. foliicola Woronich.); 20-borax Spotting (o4-M. Necans Ferr.); 21-monilialnyj Burn, monilios (o5-M. laxaHer.= M. cinereaBonord); 22-fruit Rot, monilios (o6-Monilia fructigenaPers.); 23-Chernyak (p – Sphaeropsis malorum Peck.); 24- (s-Alternaria spp).

According to the obtained materials, all studied fruit crops were affected by leaves and shoots rust (gymnosporangium).) and powdery mildew (Podosphaeraesp.), caused by phytopathogenic fungi-obligate highly specialized parasites, biotrophs (table 1). These same genera of plants are systematically belonging to a number of pathogens being to typical facultative parasites, necrotrrophs, usually with a wide phylogenetic specialization. Monilinia fruit rot (MoniliafructigenaPers.), gray rot of flowers and fruits (BotrytiscinereaPers.), black cancer (SphaeropsismalorumPeck.) and Anthracnose, or, bitter fruit rot (Colletotrichumfructigenum (Berk.) Vassil.) are found on the fruits of all studied crops (Apple, pear, quince and medlar). And early blight (Alternariaapp.) during the growing season, not only their fruits are affected, but also their leaves. All four cultures are affected by fungi of the genus Phyllosticta, causing phyllostictiosis, or brown leaf spotting, with each of them parasitizing its own kind of pathogen, respectively. P. maliPr.et Del., P. pirinaSacc., P. cydoniaevar. Cydoniicola (Allesch.) Cif. and P. mespili Sacc. Brownish, or brown spotting, caused by Entomosporangiummaculatum Lév. f. maculate Kleb. occurs on pear and quince, and medlar affects specialized only on this culture species E.
(Diplocarpon) mespili Sacc. Fungus Ascochyta pircola Sacc. parasitizes only on the leaves of pear and Apple trees, medlar defeat causes a close species. Mespili West., no pathogens of this genus have been recorded on quince.

Analysis of the species composition of mycosis pathogens of these representatives of the subfamily Yablonevye indicates the presence in the German medlar of the largest number of highly specialized pathogens belonging to the group of facultative parasites and facultative saprotrophs. This may be due to the physiological differences of these fungi, determining their different reactions when settling the surface tissues of plants and the further development of mycelium and sporulation.

Of course, the degree of defeat of representatives of this subfamily strongly depends on varietal characteristics and conditions of their growth. But in General, according to our long-term observations, quince and medlar are more resistant to the complex of fungal diseases, wild forms of which and most varieties are practically not affected by diseases of fungal etiology.

According to our observations, the features of the structure of the integumentary tissues are of great importance at the first stages of the pathological process. The study of the leaves of Maloideae with the help of SAM showed that their surface is characterized by a number of common morphogenetic features. First of all, it is hypostomy (stomata are located only on the abaxial surface of the leaf) and anomocytic type of stomata (adjacent to the stomata cells do not differ in shape from the epidermal proper), folded microrelief of the surface, the presence of single and multicellular trichomes (Fig. 1, 2). At the same time, the abaxial and adaxial surfaces of the leaf epidermis have different microrelief specificity due to functional load. Due to its stability, including in different conditions of plant growth, the features of the microrelief of the leaf surface can be considered as diagnostic signs and used not only in the systematics and taxonomy of plants, but also as possible markers of resistance to pathogens at the stage of their penetration.

The adaxial surface of the studied representatives of the subfamily maloideae is characterized by different morphology and degree of development of the cuticular folds (Fig. 1, a-d).

However, for all four genera, a rather weak development of epicuticular wax is typical, so they are strongly affected by pathogens that easily penetrate directly through the integumentary tissues. Such pathogens include pathogens of powdery mildew (Podosphaerasp.) and rust (Gymnosporangiumsp.), widespread and quite harmful to these plants (table 1). Infection with many other fungi occurs mainly through mechanical damage to the adaxial surface.

For the abaxial epidermis of the studied representatives of maloideae, different micromorphology of the cuticle folds responsible for the regulation of stomatal movements is characteristic (Fig. 2, a-d). For example, on the leaves of quince and medlar, the folds in the stomata are arranged in the form of distinct high peristomatic rings encircling the closing cells (Fig. 2, c, d). And on the leaves of Apple and pear they are less pronounced, with numerous radial and divergent in all directions microtubules, often extending over the borders of the epidermal cells proper (Fig. 2, a, b).
Fig 1. Microrelief of the adaxial epidermis of Apple leaves (Maloideae): a-Malus Mill.; b-Pyrus L.; c-Cydonia Mill.; d-Mespilus

Fig. 2. Cuticular microtubules and peristomatic rings on the abaxial epidermis of Apple leaves (Maloideae): a-Malus Mill.; b-Pyrus L.; c-Cydonia Mill.; d - Mespilus L. the Arrows indicate: Designatio: s – stoma.

The high height of the cuticle folds changes the wettability of the leaf surface [10]. Water droplets due to high surface tension only touch the upper edges of the cuticle ridges and therefore easily roll off the epidermis. We can assume that similarly, the blowing air currents or rain of spores (conidia) of phytopathogenic fungi, having round shape, which they are unable to be held firmly on the folds of the cuticles before the process will start.
sprouting hyphae. The nature of the surface of the leaves of Cydonia and, to a somewhat lesser extent, mespilus Contribute to increased resistance to fungal pathogens at the stage of their hit on the surface of the affected organ, preventing further penetration.

The structure of stomata and their number on the leaf, also have great importance if the pathogen penetrates through them into the tissues. Narrow slits of the stomata (lenticels) with a more rare arrangement of the delay the infection of plants by fungal and bacterial infection. The feature of the stomata structure noted by us in representatives of the genus Mespilus is narrow stomatal slits with outgrowths raised above the surface (Fig. 2, d), with a high probability, complicates the penetration of pathogens.

**Fig. 3.** The surface of the abaxial epidermis of Apple leaves (Maloideae) with different pathogenic fungi: a, b-Cydonia oblonga Mill.; c, d - Mespilus germanica L.; e, f - Malus Mill. (mycelium of the fungus in the stomatal slit).
In members of the genus Pyrus, the availability of penetration of fungi through the stomata is due to the absence of these features and the depth of the stomata in the epidermal tissue (Fig. 2, b). In this case, a funnel is formed, protecting the spores from rapid removal by air or water flows in the natural conditions of plant growth.

It is also important to note that the penetration of pathogenic fungi in plants rhodarugis in many cases is carried out through the spaces between the closing and the epidermal cells themselves (Fig. 3, a, b), and not the traditional methods through the stomatal cleft, as in Malus (Fig. 3, e, f). The penetration of pathogenic fungi into the leaf tissue can also be carried out through the bases of fallen trichomes, as for example, noted by us for the genus Mespilus (Fig. 3, c, d).

4 Conclusions

It was established that representatives of the genera Malus, Pyrus, Cydonia and mespilus among phytopathogenic fungi with different types of parasitism have common pathogens, as well as highly specialized ones, more represented on Mespelusgermanica. Higher, in comparison with Apple and pear, resistance to a complex of fungal diseases is established in quince and medlar. This resistance to penetration of phytopathogens, as the initial stage of the pathological process, is associated with the morphology of stomata in the abaxial epidermis of leaves, the features of the layered structure of the cuticle, the presence of suberized cells of medlar fruits, powerful flat cuticle cover in quince, as well as a high content of polyphenols in the cells of the outer layer of their amnion.

On the example of representatives of the subfamily Maloideae, as model objects, different electron microscopic methods revealed only common to the studied genera structural features of the epidermis, such as hypostomacy, anomocytic stomata, folded microrelief, the presence of one- and multicellular trichomes, but also setrodis-specific differences in the abaxial and adaxial surface of the leaves, as well as the ultrasculpture of the fruit surface. In the studied genera, the content of condensed polyphenols in the epidermis and hypodermis of the fruits was different. In our opinion, the established genus-specific microstructural and functional features of the integumentary tissues determine the different degree of determination of passive immunity to mycoses within the subfamily Maloideae.

The priority data obtained with the use of electron microscopy methods on the relationship of surface tissue microstructure features, their functional state and fungal pathogen infestation can be useful and important in the selection work on the breeding of resistant varieties. These data are also of practical interest for assessing the physiological and phytosanitary condition of vegetating plants, as well as their products during storage to determine the rates and stages of pathogen development. Timely monitoring of the plant surface by modern microscopic methods will help to identify the primary signs of infection and determine the timing of preventive protective measures, as well as reduce the toxic load and adverse impact on the environment by reducing the use of pesticides.

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