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Anatomical Changes in Peach Leaves Infected by Taphrina deformans (Berk.) Tul.

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Abstract. Light microscope study of *Prunus persica* (L.) Batsch. (Fayette cultivar) leaf anatomical structure, naturally infected by *Taphrina deformans* (Berk.) Tul. has been conducted. In the infected leaves histological changes were observed such as increase of the total thickness of the mesophyll and a loss of its differentiation to palisade and spongy parenchyma. An increase in the size of the upper epidermis was established as a result of fungus localization. The results were supported by morphometric and statistical analysis.

Key words: leaf curl, *Prunus persica; Taphrina deformans;* leaf anatomy.

Introduction

Peach leaf curl caused by *Taphrina deformans* (Berk.) Tul. is a disease widely spread and potentially harmful in cool and humid spring seasons. Morphological traits are related to leaf deformation and changes in pigmentation (chlorotic and anthocyanic coloration) of leaves, shoots and occasionally of fruits (Fig. 1). They are due to deep physiological alterations leading to premature aging and leaf abscission. Such are the abnormalities in the leaf gas-exchange, pigment and water content, etc. (RAGGY, 1966; MONTALBINI & BUONAURIO, 1986; RAGGY, 1995; PIPERKOVA & VASILEV, 2000). Changes in the structure of the leaf blade and of the cell shape and structure were described in the papers of MARTE & GARGIULO, 1972; SYROP, 1975; HUANG *et al*., 1993; ADEKUNLE *et al*., 2005. The hormone imbalance leading to hypertrophy and hyperplasia in the infected leaves is in a causal relationship with the changes on a physiological and structural level (SZIRAKI *et al*. 1975; YMADA *et al*. 1990; TSAVKELOVA *et al*. 2006).

Using light and electron microscopic observations, SYROP (1975) and HUANG *et al*. (1993) detected similar changes in almond and peach leaves infected by *Taphrina deformans*. They pointed out that accelerated cell division was the first response of the host. Changes in the palisade tissue, deformation and decreased size of the chloroplasts in the cells surrounded by the pathogen hyphae were found out, followed

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by the development of chlorosis. BASSI *et al.* (1984), GIORDANI *et al.* (2012) proved in their studies that the mesophyll layers could not be well-defined in the leaves infected by *Taphrina deformans*. Similar results were also established in the investigation carried out by HANSEN *et al.* (2007) on *Nothofagus pumilio* leaves infected by *Taphrina entomospora*.

Similar studies have not been carried out in Bulgaria. In the present investigation the authors offer their vision on the structural and anatomical changes detected in peach leaves of Fayette cultivar.

Fig. 1. Symptoms of naturally infected by *Taphrina deformans* leaves of *Prunus persica* (L.) Batsch.

Material and methods

The samples for the analyses were prepared from peach leaves of Fayette cultivar (*Prunus persica* (L.) Batsch), naturally infected by *Taphrina deformans*, as well as healthy leaves (control), collected at the end of April 2011 in the region of Plovdiv.

Both variants were studied following the standard methods of comparative anatomy (NIKOLOV & DASKALOV, 1966; METCALFE & CHALK, 1979). The cross-sections of fresh materials were studied from the middle part of the lamina. Semi-stable microscopic preparations were made. Amplival microscope was used for the light study. Measurements were made with an eyepiece micrometer (10x) and the pictures were taken with a light digital microscope Motic DMBA-210.

Part of leaves of fully development infected and healthy leaves (control) were processed following the protocol, described by DONCHEVA *et al.* (2001). Briefly, the samples were fixed in 5% (v/v) glutaraldehyde in 0.1M Na – cacodilate buffer (pH 7) for 2 h at room temperature and post fixed with 1.3% (w/v) OsO_4 in the same buffer. Infiltration and embedding were performed using Durcupan ACM (Fluka, Sigma – Aldrich). Semi-thin section (1 -2 μm), cut from the Durcupan – embedded material with glass knives, were mounted on glass slides, stained with fuchsin and methylene blue and examined under a NU light microscope (Zeiss, Jena, Germany). Metric characteristics of the studied samples included: thickness of the adaxial (ad) and abaxial epidermis (ab) in μm, total thickness of the mesophyll in μm, thickness of the palisade and spongy mesophyll layers in μm. 30 measurements of each characteristic were obtained.

The statistical analysis of empiric data includes point and interval estimation of parameters: mean on each variable (metric statistic), and difference between means for infected and control leaves, at a 95 % confidence level. Null Hypothesis for no effect of infection is proved by a t-test for independent samples at a significance level $\alpha = 0,05$. Programs were used for data processing STATISTICA for Windows (STATSOFT INC., 2007).

Results and Discussion

Studies on the anatomical structure of the leaves are of great importance for explaining the adaptive responses of the plants to ecological or pathogenic stress.

By means of light microscope analysis of cross-sections samples of healthy peach leaves (control), it was found out that the epidermal tissue was formed by a single cell layer. The mean height of the adaxial epidermis (ad) was 14.33 µm, and of the abaxial one (ab) – 12.50 µm (Table 1). The mesophyll was of a clearly defined palisade and spongy parenchyma, i.e. dorsoventral type. That structure is typical of most dicotyledonous plants, leaves of which have a flat blade and are horizontally arranged. The palisade parenchyma is structured by oval-cylindrical cells arranged in two rows, with well-developed protoplasma organelles and small intercellular spaces for free gas-exchange, the average thickness being 50.58 µm (Table 1). The cells of the spongy parenchyma are irregular in shape, arranged in several rows, with large intercellular spaces. Its average thickness is 52.41 µm (Fig. 2; 3, Table 1). Vascular bundles are located at the border between palisade and spongy parenchyma.

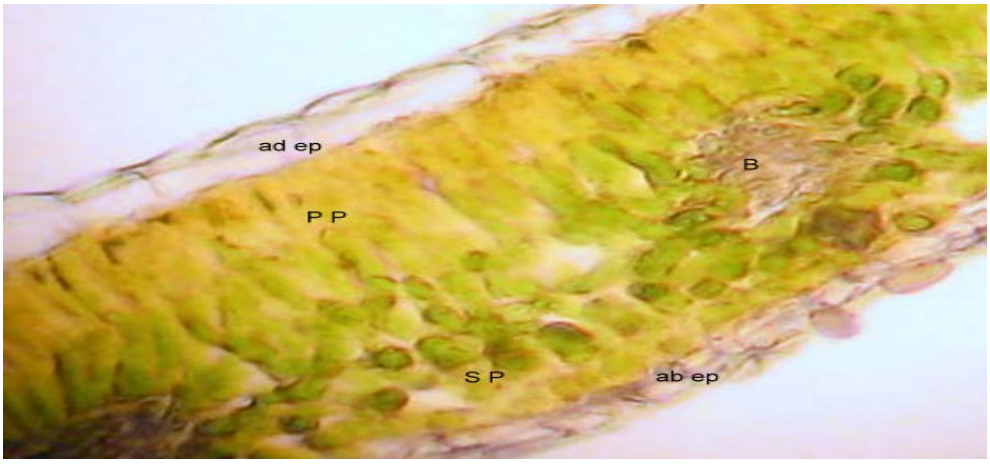

Fig. 2. General view of the transverse section of healthy leaves of *Prunus persica* (L.) Batsch. Fayette cultivar, LM at magnification X 100 (ad ep – upper epidermis; ab ep – lower epidermis; PP – palisade parenchyma; SP – spongy parenchyma).

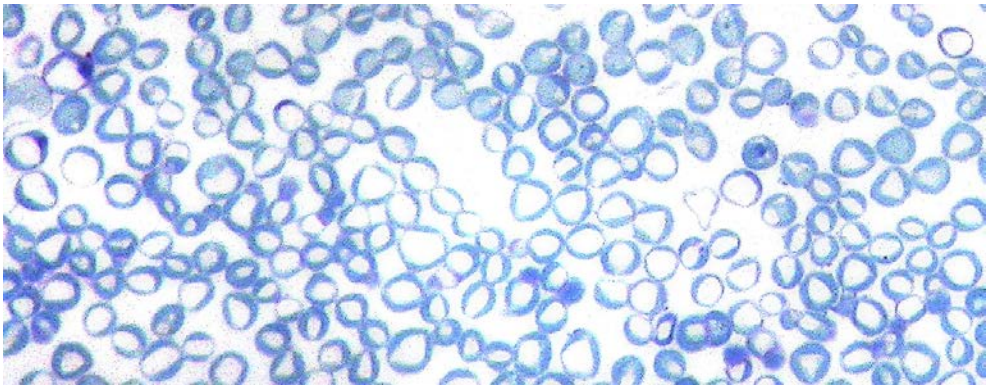

Fig. 3. Semi-thin section of healthy leaves of *Prunus persica* (L.) Batsch. Fayette cultivar, LM at magnification X 400.

As it is known, the parasitizing hyphae of *Taphrina deformans* develop in the intercellular spaces of the mesophyll and the epidermis (SYROP, 1975; GIORDANI *et al.* 2012). They cause histological changes like hypertrophy and hyperplasia, provoked by biologically active substances (SZIRAKI *et al.*, 1975; YMADA *et al.*, 1990). Statistically

significant histological changes in the leaf structure were found out in the samples prepared by infected plants. They were detected in the height of the adaxial (ad) and abaxial epidermis (ab), in the thickness of the leaf lamina, in the shape and size of the mesophyll cells. The adaxial epidermis height increased by about 45% compared to the control, i.e. by 6.50 μm in average, while the abaxial decreased from 12.50 to 10.25 μm, i.e. by about 22% (Table 1). The results obtained about the abaxial epidermis height did not agree with those provided by HANSEN *et al*. (2007) in their studies of *Nothofagus pumilio* infected by *Taphrina entomospora*. The reason for that discrepancy in the obtained results is due to the fact that the parasitizing mycelium of *Taphrina deformans* at the end of the pathological process develops subcuticularly, between the adaxial epidermis cells, while the mycelium of *Taphrina entomospora* develops on the abaxial epidermis. Accordingly, the asci are formed on the upper leaf surface in peach and on the lower one in *Nothofagus pumilio*. It could be admitted that thickening of the epidermal layer, spores of the pathogen are bursting through, is a protective mechanism of the host.

Disrupted mesophyll differentiation was established in the areas with parasitizing mycelium, i.e. the leaf lamina changed from dorsoventral to isolateral (equifacial) (Fig. 4.). In result of the processes of hypertrophy and hyperplasia, provoked by the pathogen, the shape of the cells from the palisade layer turned from cylindrical oval to isodiametric. Both, large hypertrophied, strongly vacuolated cells, with organelles located close to the cell wall, with small intercellular spaces were observed, together with cells twice or thrice smaller in size (Fig. 4.).

The morphometric analysis of the cross-section of the control and of the infected leaves confirmed the histological changes (Table 1). The total average thickness of the mesophyll in the first variant was 120.92 μm, while in the infected leaves it reached up to 197.50 μm. The registered difference of 76.58 μm is statistically significant.

The established changes in the structure of the leaf lamina correlated with some changed parameters of the physiological state of the peach leaves infected by *Taphrina deformans*, to which Fayette cultivar is susceptible. The chlorophyll content decreased by 22-29%, the photosynthesis rate was inhibited in the range of 17 to 45% and breathing increased repeatedly (PIPERKOVA & VASILEV, 2000). That confirmed the fact that the histological changes established in the present study by light microscopy, as well as the changes in peach cultivars showing different resistance to the pathogen, found out by GIORDANI *et al*. (2012), are causally related to the disturbed metabolism in the infected leaves.

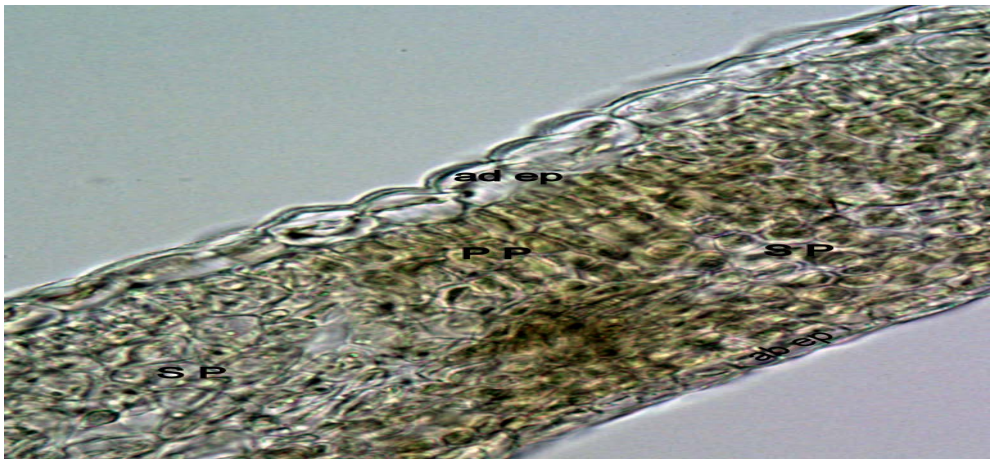

Fig. 4. General view of transverse section of infected by *Taphrina deformans* leaves of *Prunus persica* (L.) Batsch. LM at magnification X 100 (ad ep – upper epidermis; ab ep – lower epidermis; spongy parenchyma.

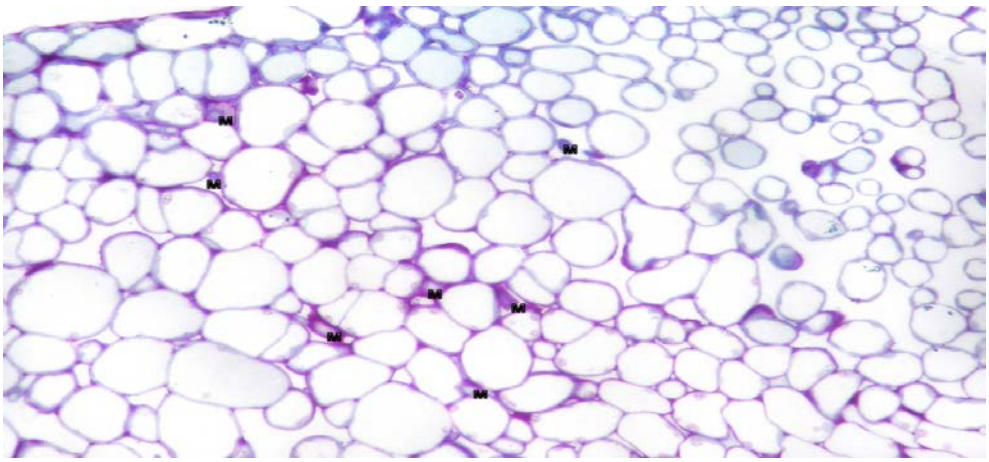

Fig. 5. Semi-thin section of infected by *Taphrina deformans* leaves of *Prunus persica* (L.) Batsch. LM at magnification X 400 (M – mycelium).

Table 1. Morphometric parameters of healthy (control) and infected by *Taphrina deformans* (Berk.)Tul. leaves of *Prunus persica*(L.) Batsch.

Parameters		95 % Confidence Interval		
		Mean	Standard Deviation (SD)	Difference between Means
Upper epidermis (ad), thickness/µm	control	14.33 ± 0.65	3.9108	6.50 ± 0.87*
	infected	20.83 ± 0.62	21.3247	
Lower epidermis (ab), thickness/µm	control	12.50 ± 0.60	1.7287	-2.25 ± 0.84*
	infected	10.25 ± 0.62	1.6522	
Mesophyll thickness, µm	control	120.92 ± 1.46	1.6082	76.58 ± 8.07*
	infected	197.50 ± 7.96	1.6544	
Palisade mesophyll, thickness/µm	control	50.58 ± 1.09	2.9127	-
Spongy mesophyll, thickness/µm	control	52.42 ± 1.44	3.8553	-

*statistically significant difference at α = 0.05

Conclusion

In result of the study carried out with light microscopy on healthy and naturally infected by *Taphrina deformans* peach leaves (*Prunus persica* (L.) Batsch.) of Fayette cultivar, severe histological changes were established. The leaf structure changed considerably from dorsoventral to isolateral. Under the influence of the processes of hypertrophy and hyperplasia, the plant cells located around the fungal hyphae, were strongly vacuolized, they increased in size and acquired an isodiametric shape. The morphometric analysis confirmed the increase of the adaxial epidermis height by 45% compared to the control and the decrease of the abaxial epidermis height by 22%, which was caused by the *Taphrina deformans* asci formed on the upper surface of the peach leaves. The established changes were statistically significant. Structural differences in the infected leaves were directly related to the changed physiological state of the plant.

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