Histological differences between the leaves of spring and winter ecotypes of poppy (*Papaver somniferum*) varieties

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Abstract

In this study it was sought for histological differences between the spring and winter ecotype of poppy varieties, under standard condition. The thickness of epidermis cells and the cuticle of leaves were measured under light microscope. It was observed that the overwintering genotypes had significantly thicker cuticle than the spring varieties, on both the abaxial and the adaxial sides of the leaves. Regarding the upper and the lower epidermis, differences between varieties were not found. Cuticle thickness may be related to cold tolerance and offers the possibility of a rapid screening for new winter poppy genotypes, what may facilitate the further breeding process.

Key words: epidermis, cuticle, abaxial, adaxial

Introduction

Poppy (*Papaver somniferum* L.) is an important horticultural plant, because it not only has culinary values, but is also a medicinal plant – its capsule is utilized by pharmaceutics and its seeds by food industry. Recently, two ecotypes of poppy are cultivated: spring ecotype (i.e. frost sensitive varieties) and winter poppy (frost tolerant varieties). In Hungary, both winter and spring ecotypes of poppy are grown on more than ten thousand hectares. In the Central-European region, a growing interest can be observed for winter poppy varieties, because of their higher and more stable yields. Although spring ecotypes possess enhanced frost tolerance in the leaf rosette stage, these varieties are generally killed or severely damaged by the winter frosts. Breeding has been started to establish new, frost tolerant genotypes. Although former studies suggested that chiefly soluble sugar (glucose, fructose, sucrose) accumulation can be the marker of the tolerance, such histological adaptations that can be related to the cold tolerance of winter poppy varieties would be useful for a rapid screening for new winter poppy genotypes and facilitate the further breeding work.

It is well known that physiological and morphological changes eventuate in plants grown at low temperatures (Boese and Huner, 1990). In case of winter cultivars of wheat Equiza et al. (2001) observed relatively smaller root system, lower stomatal frequency and increased epidermal cell wall thickness at low temperature (5°C), relative to the spring cultivars. Stefanowska et al. (1999, 2002) found in case of winter oilseed rape, that low temperature results changes in the dimension of mesophyll cells and increase in the thickness of leaf cell walls during cold acclimation.

The histological background of cold stress adaptation of the poppy is poorly understood. Dai et al. (2004) have analysed the main anatomical and surface characteristics of leaves of *Papaver croceum* from high altitude cold habitats (at geographical elevation of 3600 m and 2500 m). According to their results on this species, the individuals living at different elevations showed difference concerning their leaf thickness, the shape of the epidermis cells, the stomatal size and the stomatal density. Rahmatpour et al. (2010) compared several species of wild poppy in virtue of morphological and anatomical adaptations. Their results indicated significant difference in epidermis and cuticle thickness and confirmed the variability of the species according to their habitats.
Investigating the histological differences between the ecotypes of different varieties is not a searched issue, thus it is poorly understood. The aim of our study was to find anatomical differences between the winter and spring ecotypes of poppy, in order to enhance the breeding in the future in the process of screening for cold resistant varieties.

**Materials and methods**

**Sample taking**

The examination was carried out at the research field of the Department of Medicinal and Aromatic Plants of Corvinus University of Budapest, in 2011. The experimental area is located in the south-eastern part of Budapest, at geographical elevation of 100-150 m. The soil is light sand, with low (0.2-0.4%) organic content, humus content is 1.5%, nitrogen and phosphorous levels are medium, it is properly supplied with potassium.

The experimental genotypes of poppy were as follows (all registered varieties): 'Ametiszt', 'Medea', 'Korona', 'Tebona' varieties represented the spring ecotype and 'Zeno', 'Kozmosz', 'Leila' cultivars of the winter poppy. In each plot, seeds were sown by hand into open field at the end of September 2010. The plots were of 10 m² extension, and 3 replications were made. During the vegetation period, the usual poppy agrotechnics was applied. Samples were collected on 23th May, 2011.

**Microscopic analysis and statistics**

Leaves were cross sectioned at the basal portion in the lamina (through the midrib) using a freezing microtome (Leitz Wetzlar). For the measurements all the chosen leaves were of the same stage of maturity. The anatomical features of the 25-30 μm thick sections were studied under a light microscope (Zeiss, Axio Imager A2) with dark-field illumination. Thickness of adaxial and abaxial epidermis cells and cuticle thickness of both epidermis layers were measured. All the measurements and the image documentation were carried out with the Axion Vision 4.8 software. For each variety 30 different measured data of the leaves were documented.

Descriptive statistics, multivariate of variance analysis and t-tests were calculated with the PASW Statistics 18 software.

**Results and discussion**

**Epidermis thickness of the poppy varieties**

Among the spring ecotype varieties 'Ametiszt' had the thickest (37.4 μm), and 'Tebona' had the thinnest adaxial epidermis (33.7 μm) (Figure 1). According to the statistics 'Ametiszt' differed significantly from the others, while the other three varieties did not differ. In case of the abaxial epidermis other trends were found. 'Medea' had significantly the thickest epidermis (37.8 μm) compared to the others, while 'Korona' had the thinnest one (31.6 μm). Nevertheless, only 'Medea' had thicker epidermis on the abaxial than on the adaxial side (Figure 1).

![Figure 1. The thickness of adaxial and abaxial epidermis of the different varieties](image-url)
Histological differences between the leaves of spring and winter ecotypes of poppy (*Papaver somniferum*) varieties

In case of winter poppy varieties the thickness of adaxial epidermis changed as follows: 'Zeno' had the thinnest epidermis (31.4 μm) and 'Leila' had the thickest one (32.8 μm). The latter difference was proven as significant by the statistical analysis. 'Leila' had the thickest abaxial epidermis also and 'Kozmosz' had the thinnest one, but there weren’t any significant difference between them (Figure 1).

**Cuticle thickness of the poppy varieties**

Among the varieties of the spring ecotype, 'Tebona' had significantly the thickest adaxial cuticle (6.9 μm), while the thinnest cuticle was observed in case of 'Medea' (5.0 μm) (Figure 2). Regarding the abaxial cuticle, 'Korona' had the thickest (8.6 μm) and 'Ametiszt' the thinnest (5.9 μm) cuticle, with a statistically proven significant difference between them. In case of each variety, the abaxial cuticle was significantly thicker than that of the adaxial side.

![Figure 2. The thickness of adaxial and abaxial cuticle of the different varieties](image)

<table>
<thead>
<tr>
<th>Variety</th>
<th>Adaxial Cuticle</th>
<th>Abaxial Cuticle</th>
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<tbody>
<tr>
<td>'Ametiszt'</td>
<td>* * * * *</td>
<td>* * * * *</td>
</tr>
<tr>
<td>'Medea'</td>
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<td>* * * * *</td>
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<tr>
<td>'Korona'</td>
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<td>*</td>
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<tr>
<td>'Tebona'</td>
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<td>*</td>
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<tr>
<td>'Zeno'</td>
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<tr>
<td>'Kozmosz'</td>
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<tr>
<td>'Leila'</td>
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</table>

In case of the winter poppy varieties, the thickest adaxial cuticle was found on 'Leila' (8.8 μm) and 'Zeno' had the thinnest one (6.5 μm). Concerning the abaxial side, ‘Kozmosz’ had the thickest (10.7 μm) and ‘Zeno’ had the thinnest cuticle (6.5 μm) (Figure 2, Table 1).

**Comparison of spring and winter ecotypes**

Considering the mean values of both ecotypes, calculated from all measured data of the varieties it was found that the mean thickness of adaxial and abaxial epidermis (35.3 and 33.5 μm, respectively) appear to be thicker in case of the spring ecotype compared to the winter poppy (33.6 and 31.1 μm). However, the statistical analysis did not prove significant difference (Table 2). Nevertheless, in case of cuticle thickness...
significant difference was found between spring ecotype and winter poppy varieties, both on the adaxial and the abaxial side. The abaxial cuticle was thicker (9.6 μm) than the adaxial one (7.6 μm).

Accordingly, cuticle thickness can be a predictive characteristic of frost tolerant genotypes. On the contrary, thinner cuticle is not necessarily related to intolerance, because the winter poppy ‘Zeno’ did not differ significantly from the spring ecotype varieties: ‘Tebona’ and ‘Korona’ (Figure 2, Table 1).

Table 2. The mean values of epidermis and cuticle thickness of different ecotypes (μm)

<table>
<thead>
<tr>
<th>Ecotypes</th>
<th>Adaxial Epidermis</th>
<th>Adaxial Cuticle</th>
<th>Abaxial Epidermis</th>
<th>Abaxial Cuticle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Dev.</td>
<td>Mean</td>
<td>Std. Dev.</td>
</tr>
<tr>
<td>Spring ecotype varieties</td>
<td>35.3 A</td>
<td>7.6</td>
<td>5.7 A</td>
<td>1.2</td>
</tr>
<tr>
<td>Winter poppy varieties</td>
<td>33.6 A</td>
<td>7.8</td>
<td>7.6 B</td>
<td>1.4</td>
</tr>
</tbody>
</table>

The leaves of *Papaver somniferum* L. adapt to environmental conditions, thus anatomical changes appeared in tissue structure of the mesophyll as well as of the epidermis. Environmental effects (e.g. light) affect the leaves from both sides, whereby abaxial epidermis appear to be similar to the adaxial epidermis, while the mesophyll of poppy leaves is isolateral. It is in agreement with Stefanowska et al. (1999, 2002), who found changes as well in the dimension of mesophyll cells of winter oilseed rape resulted by low temperature.

Conclusions

Significant difference between the cuticle thickness in varieties of spring ecotype and winter poppy were observed, which means that the genotypes able to overwinter have thicker cuticle than the spring varieties. This difference occurred both on the abaxial and the adaxial sides of the leaves, as well. However, regarding the epidermis thickness, there was no statistically proven deviation between the ecotypes. It can be concluded that cuticle thickness may be related to cold tolerance and as a predictive adaptation offers the possibility of a rapid screening for cold tolerant new winter ecotypes of poppy and facilitate the further breeding work.

References


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