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Leaf Anatomical Structure in *Arabidopsis thaliana* (L.) Heynh. Mutants Deficient in Photoreceptors.

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ABSTRACT

Light is one of the major environmental factors affecting plants growth and development. For the reception of light signals, photoreceptors are used, activating complex signaling pathways which modulate plant's life strategy. The isolation of individual or multiple photoreceptor-deficient plant mutants provided valuable insights into photoreceptors functions, including the effects on plant architecture. However, available information on the leafanatomy in such mutants is very limited. Here, we characterized leaf anatomical structure of model plant *Arabidopsis thaliana* (L.) Heynh. Individual mutants deficient in phytochromes, cryptochromes, and phototropin were compared to the wild type control variant. We analyzed the patterns of distribution of different tissues and vascular bundles in the leaves and calculated the total leaf areas, vascular bundles and individual tissues areas, as well as leaves thickness. The results indicated asignificant increase in tissue areas in the mutants*cry1* and *phot1*, while in *phyB* mutants, the leaf lamina was slightly underdeveloped and smaller than that of the control Lervariant.In *cry2*mutant, leaf area increased slightly, and in *phyA* somewhat larger leaves exhibited less developed central vascular bundle. Thus, the deficiency in blue or red light perceiving receptors might have an effect on the leaf structure in *Arabidopsis thaliana*, which is consistent with the previous observations reported elsewhere.

Keywords: *Arabidopsis thaliana*; mutants; photoreceptors; leaf anatomical structure; leaf area.

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INTRODUCTION

To optimize growth and development, plants have to respond to the signals of surrounding environment. For perception and transformation of signals from various environmental factors, such as light, temperature, and humidity, plants use a wide range of signaling systems [1-4].As one of the main environmental factors, light is not only a source of energy for plants growth, but it also provides information on the location andmodulates many developmental processes, including seed germination, flowering, de-etiolation, phototrophic and photoperiodic reactions, and circadian clock [5-9].For the reception of light signals, photoreceptors are used. Three major families of photoreceptors arerecognized: phytochromes, cryptochromes, and phototropins [10-12].

Phytochromes absorb light in red (R) and far-red (FR) spectrum (600-750 nm). Biologically inactive R-adsorbing phytochromes are synthesized in dark and are converted to an active FR-adsorbing form at R light. Under FR light, the reverse conversion occurs, which keeps the system in photoequilibrium in natural conditions. This photoreversibility assay is the primary mechanisms in the signaling pathway which regulates gene expression in response to the light changes [5,13]. In *Arabidopsis thaliana*, five genes (*PHYA*-*PHYE*) encoding phytochromes have been identified [14,15]. The isolation of individual or multiple phytochromes-deficient mutants provided valuable insights into phytochromes functions, including the effects on plant architecture. In *Arabidopsis thaliana* mutants deficient in one or multiple phytochromes, changes in plant architecture included elongation of petioles, leaf elongation, reduced leaf area, loss of rosette habit [5,13,16,17].For rice (*Oryza sativa* L. cv. Nipponbare) *phyB* mutants, reduced leave area and larger epidermal cells have been also reported [18].

Two other types of receptors, cryptochromes and phototropins, absorb blue (B) and UVA (320-500 nm) light.In *Arabidopsis thaliana,* three genes encoding cryptochromes (*CRY1, CRY2*, and *CRY3*)and two genes encoding phototropins (*PHOT1* and *PHOT2*) have been identified [19]. Cryptochromes regulate photomorphogenic responses, while photochromes are involved in regulating photo-induced movements [12].Blue light was shown to have an effect on soybean isolineleaf area[20]. The study on *Arabidopsis thaliana* mutants deficient in blue-light receptors indicated that light quantity has an effect on leaf mesophyll cell development [21].

The available information on leaf anatomy in plant mutants deficient in photoreceptors is still very limited. The aim of this study was to characterize anatomical structure of leaves in individual *Arabidopsis thaliana* mutants deficient in phytochromes, cryptochromes, and phototropin. Leaves' structural overviews of the *phyA*, *phyB*, *cry1*, *cry2*, and *phot1* mutants were compared to the control variant Ler.

METHODS

The leaves of the following *Arabidopsis thaliana* mutants were inspected:*phyA*[22], *phyB*[23], *cry1* [24],*cry2*[25], and *phot1*[26]. The leaves were provided by Jagiellonian University, Faculty of Biochemistry, Biophysics and BiotechnologyDepartment of Plant Physiologyand Biochemistry (Krakow, Poland).The plants were grown in the controlled environment (greenhouse, 22 °C) under artificial lighting.

The leaves of each sample were fixed in Chamberlain solution and further processed by conventional cytological methods. The sections were preparedwith a sliding microtome MC-1 and placed on glass slides using a protein and stained withhematoxylin[27]. For each of the five mutants and a control variant,35 cross sections were inspected.For all 210 sections, the following parameters were determined: the total leaf area, the area of cancellous and columnar parenchyma, the area of epidermis (top and bottom), the area of the central vascular bundle, and the average thickness of the leaf near the midrib.

The areas of tissues were determined in two ways. In the first method, the overlay grid was used, and the area (S_t) of the tissue was calculated with the formula:

$$S_t = Z \cdot S_{sq} \cdot N \cdot \sum s_i \,,$$

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whereS_{sq} – the area of one square box in the overlay grid;N – the number of boxes covered by the observed object; s_i – the area of the object in one box ($0<s_i\leq1$; $i=1,\ldots,N$); Z=1/52,5 – the value reciprocal to the microscope magnification.

The second method involved the graphics system «KOMPAS» [28]. The object of interest was depicted with the tool “Bezier curve”, and the area of thisobject was calculated (S_T). In the same scale, the control object was depicted, and its area was also calculated (S_C). The real area of the control object was known(S_c). The area of the object of interest (S_t) was calculated with the formula:

$$S_t=S_T\cdot S_o/S_C\ .$$

The latter method showed higher accuracy, compared to the use of overlay grid. Thus, the results obtained with the“KOMPAS” system were used for further statistical processing (the programs "Excel" and "Statistika")and subsequent analysis.

RESULTS

Leave structure in *Arabidopsis thaliana* control variant (Ler)

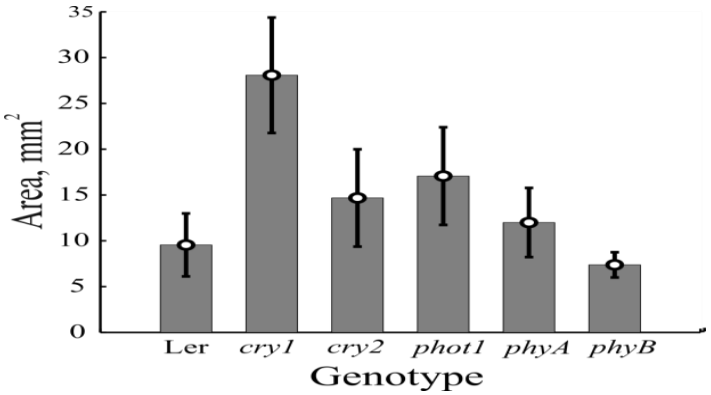

Figure 1: The total leaf area in the studied mutants and control variant (Ler), mm^2

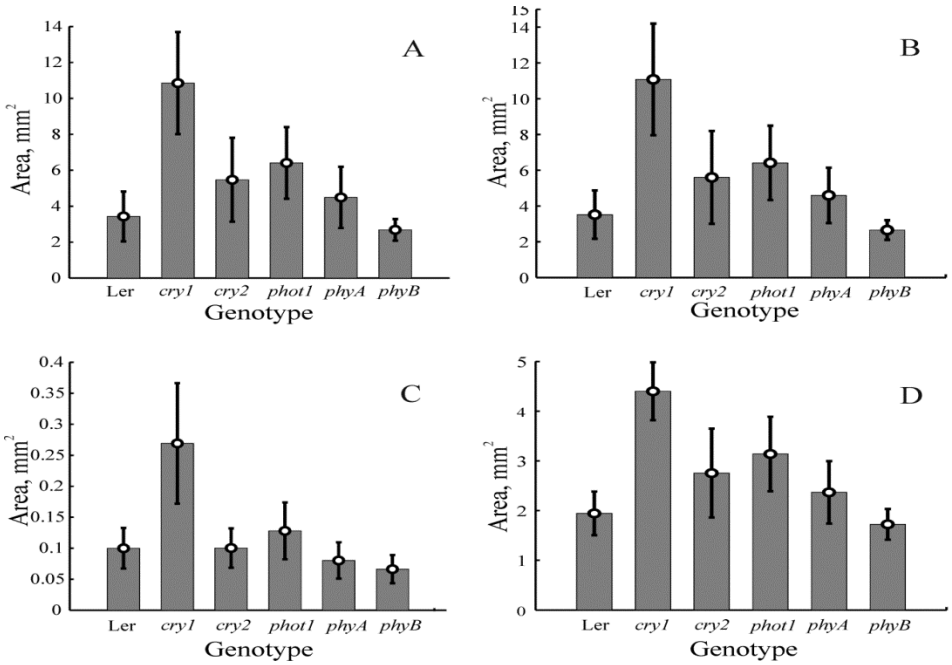

Figure 2: The area of cancellous parenchyma (A), columnar parenchyma (B), central vascular bundle (C), and epidermis (D) in the studied mutants and control variant (Ler). Error bars indicate standard deviation.

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Anatomicalelements ofparenchymal typedominate in*Arabidopsis thaliana* leaf structure. In control variant (Ler), mesophyll is divided into single-layer columnar and very loosecancellous, with lots of intercellular spaces. The vascular system is well developed and consists of 13 vascular bundles which are located along the entire length of the lamina. The upper and lower epidermis is of approximately equal thickness andis not covered by a cuticle layer.Mechanical tissue fibers are located around the vascular bundles.

In Ler, the following standard parameters were identified: the average area of the leaf was 9.54 mm² (Fig. 1),the average area of cancellous tissue was 3.43 mm² (Fig.2 A), the average area of columnar tissue was 3.52 mm² (Fig. 2 B),the average area of the central vascular bundle was 0.1 mm² (Fig.2 C), and the average area of upper and lower epidermis was 1.94 mm² (Fig. 2 D). In percentage, the areas of the central vascular bundle, columnar mesophyll, cancellous mesophyll, and epidermisaccounted for 1.13%, 36.5%, 35.49%, and 22.06%, respectively. The average thickness of the leaf near the midrib was 0.11 mm (Fig. 3).

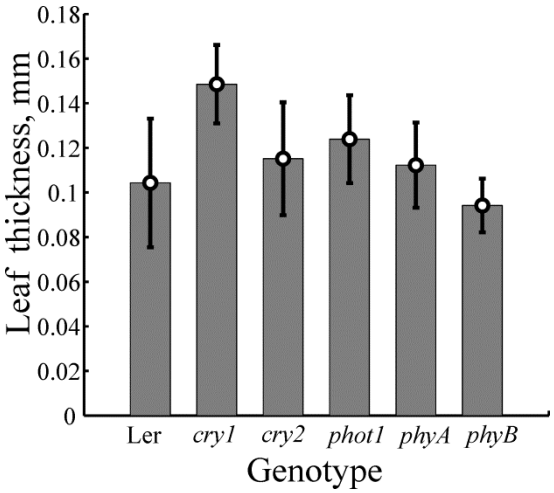

Figure 3: The leaf thickness in the studied mutants and control variant (Ler).Error bars indicate standard deviation.

Leave structure in the mutants deficient in photoreceptors perceiving blue light

Leave structure in *cry1*mutant

The average parametersfor the *cry1* mutant were the following: the total area of the leaf was 28.07 mm² (Fig. 1), the area of cancellous mesophyll was 10.85 mm² (Fig. 2 A), and columnar mesophyll area was 11.08 mm² (Fig. 2 B), the central vascular bundle area was 0.27 mm² (Fig. 2 C), and the upper and lower epidermis area was 4.4 mm² (Fig. 2D). In percentage, the areas of the central vascular bundle, columnar mesophyll, cancellous mesophyll, andepidermis accounted for 1%, 39.19%, 38.63%, and 16.1%, respectively. The average thickness of the leaf near the midrib was 0.15 mm (Fig. 3).

The length of the leaves in *cry1* mutants increased significantly (2.94-fold), compared to the control variant Ler. In vascular system,the number of fibro-vascular bundles has increased to 16, since the area of the laminaincreased. No significant changes in the anatomy of other tissues were detected.

Leave structure in *cry2* mutant

For *cry2*, the averages were: the total area of the leaf was 14.67 mm² (Fig. 1), the area of cancellous mesophyll was 5.47 mm² (Fig. 2 A), and columnar mesophyll area was 5.6 mm² (Fig. 2 B), the central vascular bundle area was 0.1 mm² (Fig. 2 C), and the epidermis area was 2.75 mm² (Fig. 2D). In percentage, the areas of the central vascular bundle, columnar mesophyll, cancellous mesophyll, and epidermis accounted for 0.77%, 37.33%, 37.02%, and 19.09%, respectively. The average thickness of the leaf near the midrib was 0.11 mm (Fig. 3).

Leaves of these mutants had a larger size compared with the control (1.54-fold). No other anatomical changes were detected.

Leave structure in *phot1* mutant

The average parametersfor the *phot1* mutant were the following: the total area of the leaf was 17.05 mm² (Fig. 1), the area of cancellous mesophyll was 6.41 mm² (Fig. 2 A), and columnar mesophyll area was 6.41 mm² (Fig. 2 B), the central vascular bundle area was 0.13 mm² (Fig. 2 C), and the epidermis area was 3.14 mm² (Fig. 2D). In percentage, the areas of the central vascular bundle, columnar mesophyll, cancellous mesophyll, and epidermis accounted for 0.85%, 37.46%, 37.84%, and 19.02%, respectively. The average thickness of the leaf near the midrib was 0.13 mm (Fig. 3).

The total leaf area in *phot1* has increased compared to the control (1.79-fold). In the tissues, no major changes were observed.

Leave structure in the mutants deficient in photoreceptors perceiving red light

Leave structure in *phyA* mutant

For *phyA*, the averages were: the total area of the leaf was 11.99 mm² (Fig. 1), the area of cancellous mesophyll was 4.48 mm² (Fig. 2 A), and columnar mesophyll area was 4.59 mm² (Fig. 2 B), the central vascular bundle area was 0.08 mm² (Fig. 2 C), and the epidermis area was 2.37 mm² (Fig. 2D). In percentage, the areas of the central vascular bundle, columnar mesophyll, cancellous mesophyll, and epidermis accounted for 0.69%, 38.17%, 36.71%, and 20.25%, respectively. The average thickness of the leaf near the midrib was 0.12 mm (Fig. 3).

The total leaf area in *phyA* has slightly increased (1.26-fold) compared with the control plants. However, the central vascular bundle was less developed (1.25-fold).

Leave structure in *phyB* mutant

The average parametersfor the *phyB* mutant were the following: the total area of the leaf was 7.36 mm² (Fig. 1), the area of cancellous mesophyll was 2.68 mm² (Fig. 2 A), and columnar mesophyll area was 2.65 mm² (Fig. 2 B), the central vascular bundle area was0.07 mm² (Fig. 2 C), and the epidermis area was 1.72 mm² (Fig. 2D). In percentage, the areas of the central vascular bundle, columnar mesophyll, cancellous mesophyll, and epidermis accounted for 0.91%, 36.04%, 36.41%, and 23.6%, respectively. The average thickness of the leaf near the midrib was 0.11 mm (Fig. 3).

The leave lamina of *phyB*mutantswas narrow and small, and the conductive system was somewhat underdeveloped (1.43-fold less than the control), which was reflected in the reduction of the central vascular bundle area (Fig. 2 C). The total leaf area was 1.3-fold less than that in the control group.

Statistical validation

The resulting statistics showed that the sample obeys the normal distribution, since the values of the mean and the median are close. T-test is in the area of significance (T> 2.65), data is valid.

CONCLUSIONS

The largest differences compared to the control group (Ler)were observed in *cry1* and*phot1*mutants: the leaves areas had significantly increased. In *phyB*mutants, on the contrary, the leave lamina was smaller and not fully developed, compared to the control variant. In *cry2* and *phyA*mutants,the tissues area washigher1.54- and 1.26-fold, respectively, than that in the control group. Based on the total leaf area, all the studied genotypes can be listed in the following descending order: *cry1*, *phot1*, *cry2*, *phyA*, Ler, and *phyB*.

Thus, in the mutants deficient in perceiving blue light of the spectrum, the increase in the total leafarea is observed. However, the violation of red light absorbing photoreceptors is, on the contrary, expressed in the decrease in leave area. The results reported here are consistent with the previous observations of architectural changes in *Arabidopsis thaliana* mutants deficient in photoreceptors, including leaf elongation and changes in leaf area [5,13,16,17,21].

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