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MORPHOLOGICAL AND PHYSIOLOGICAL PARTICULARITIES OF HOSTA LEAVES VARIETIES CULTIVATED IN ROMANIA

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Abstract

The paper presents the results of the morphological characteristics of Hosta leaves (leaves area, the perimeter, the length of the leaves, width of leaf, the surface of the dominant colour) and physiological studies (photosynthesis, respiration, transpiration and chlorophyll) got from 'T. Rex', 'American halo', 'White feather' and 'Christmas island' varieties of Hosta cultivated in "I. Todor" Botanical Garden within the University of Agronomic Sciences and Veterinary Medicine of Bucharest. The intensity assessment of the photosynthesis, respiration and transpiration was determined with the LCPro+ device, directly into the experimental field. The intensity of photosynthesis varied between 2.33 µmol CO_2 m^{-2} s^{-1} ('American halo') and 22.08 µmol CO_2 m^{-2} s^{-1} ('Christmas island'), intensity of transpiration 2.54 mmol H_2O $m^{-2}sec^{-1}$('American halo') and 6.12 mmol H_2O $m^{-2}sec^{-1}$ ('Christmas island'), intensity respiration 2.81 µmol CO_2 m^{-2} s^{-1} ('White feather') and 6.55 µmol CO_2 m^{-2} s^{-1} ('Christmas island'). The intensity of physiological processes varied depending on the light intensity, temperature and species. The photosynthetic pigments content was determined using 80% acetone extraction and by spectophotometry at wavelengths of 470 nm, 663 nm and 646 nm.

Key words*: chlorophyll, spectophotometry, photosynthesis, respiration, transpiration.*

INTRODUCTION

Hosta, also known as "Autumn lily", originates from Japan, China and Korea and it was first introduced in Europe in the late 1700s and in the US in the mid-1800s (Greenfell & Shadrack, 2004). Dr. Engelbert Kaempfer was the first man ever to see, draw and describe a Hosta, later identified as *Hosta lancifolia*. Hosta has become one of the best-selling herbaceous flowers in the world (Greenfell, 1996).

The number of currently known species is about 43 and of cultivars over 2500 (Șelaru, 2007). The most widespread species are: *Hosta plantaginea* Aschers. (syn. *Funkya subcordata* Spreng.*), Hosta sieboldiana* Engl. (syn. *Hosta glauca* Stearn.*), Hosta fortunei* (Hort.) Engl.*, Hosta undulata* (Hort.), *Hosta lancifolia* Engl. (syn. *Funkia japonica* Voss.) și *Hosta albo-marginata* Hook. (syn. *Hosta sieboldii* Aschers.) (Toma, 2009). All these species are decorative outside the flowering season as well, due to the general aspect of the plant and to the characters of the leaves (Toma, 2009). Hosta is part of the *Asparagaceae* family and is a perennial, herbaceous plant, having different sizes, from miniature species to giant species. It is considered a decorative plant mainly for its leaves in various sizes, shapes and colours, but also for its delicate flowers. The leaves may be green, blue, yellow, golden or white and may be one colour or variegated. Most varieties of Hosta show colours of flowers from purple to white.

They are the best-selling perennials in the USA and are popular in Europe and Japan, and therefore have considerable economic importance (Zonneveld & Pollock, 2012). Yu et al. (2016) indicate that nitrogen metabolism regulation, photosynthesis and energy supply, chloroplast development, and chloroplast protein import/processing play crucial roles in leaf colour changes in variegated leaves. The results provide novel insights into understanding the mechanisms of leaf colour regulation in variegate leaves. Yoshioka et al.

in 2009 found that the rate of photosynthesis (on an area basis) of the variegated leaves increased almost linearly according to the increase in the proportion of green area to total leaf area. In contrast, dark respiration rate was nearly constant irrespective of the extent of leaf variegation.

MATERIALS AND METHODS

The plants taken into study were cultivated in the "I. Todor" Botanical Garden of the University of Agronomic Sciences and Veterinary Medicine of Bucharest. The morphological and physiological characteristics were analysed in 4 varieties: Hosta cv. *T. Rex*, with green leaves, Hosta cv. *American halo*, with green-blue leaves having streaked, irregular, ivory-white edges, Hosta cv. *White feather*, with ivory-white leaves that turn to green in June due to the bright sunlight, and Hosta cv. *Christmas island*, with leaves having white color in the middle and dark green edges.
In order to determine the morphological characteristics of the leaves, 3 leaves were harvested from each of the 4 cultivars and varieties of Hosta, in July 2019. The leaves were washed with water, dried and scanned using a scanner Epson Expression 11000XL and after analysed with the WinFolia Software. The determinations done are the leaf area (cm^2), the perimeter (cm), the length (cm) and the width (cm) of the leaves (Figure 1) and the percentage of the dominant and secondary colours of the leaves. The variants were noted with V_1 - Hosta cv. *T. Rex*, V_2 - Hosta cv. *American halo*, V_3 - Hosta cv. *White feather*, V_4 - Hosta cv. *Christmas island.*
The determination of the intensity of photosynthesis, transpiration and respiration was performed directly on the green part of leaves, in the field of experience, using the LCPro+ equipment, according to Lascu et al. (2019). Triplicates of independent determinations were reported.
The quantitative analysis of the assimilating pigments was performed through the Arnon spectrophotometric method, which is based on the extraction of pigments in an organic solvent (80% acetone) and measuring the absorbance of the extract, by reading the sample extinction at a spectrophotometer at three different wavelengths: 470 nm, 646 nm and 663 nm, according to Asănică et al. (2017). In the case of the varieties with variegated leaves, the determinations were done both on the whole leaf and separately, on the green and white parts.

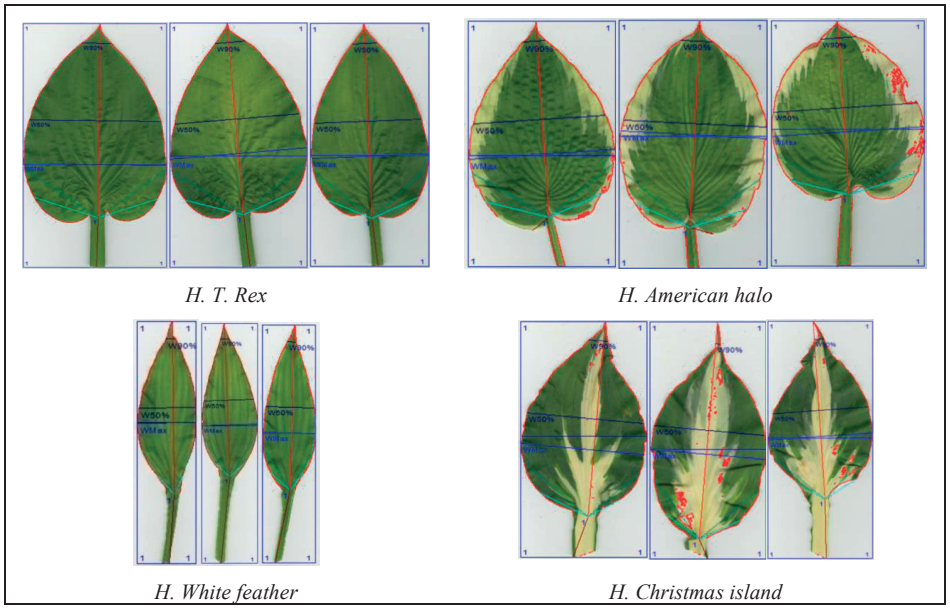

Figure 1. Morphological characteristics of the plant leaves for *Hosta T.Rex, American halo, White feather* and *Cristmas island*

The samples (consisting in the green part of the leaf, the white part of the leaf and the whole leaf) are marked with S_1 - S_8, as follows:
S_1 - *T. Rex,* the whole leaf
S_2 - *American halo,* the white part of the leaf
S_3 - *American halo,* the green part of the leaf
S_4 - *American halo,* the whole leaf
S_5 - *White feather,* the whole leaf
S_6 - *Christmas island,* the white part of the leaf
S_7 - *Christmas island,* the green part of the leaf
S_8 - *Christmas island,* the whole leaf.
Independent extract solutions were analysed in triplicate. Statistical data processing was done by Microsoft Office Excel 2013.

RESULTS AND DISCUSSIONS

From the data regarding the biometric measurements presented in Table 1 and Figure 2 it can be observed that the average leaf area has varied from 40.87 cm (V_3 - *White feather*) to 183.19 cm (V_1 - *T. Rex*).

Table 1. Biometrical measurements of the leaves

Var.	Leaf Area (cm^2)	Perimeter (cm)	Length (cm)	Width (cm)
V1	183.19±24.08	52.87±2.77	24.50±1.45	13.19±1.33
V2	181.82±31.23	64.08±2.01	22.91±2.08	13.79±1.60
V3	40.87±9.19	31.51±3.48	20.12±2.40	4.59±0.70
V4	83.29±4.39	57.44±20.0	18.40±2.43	8.87±0.49

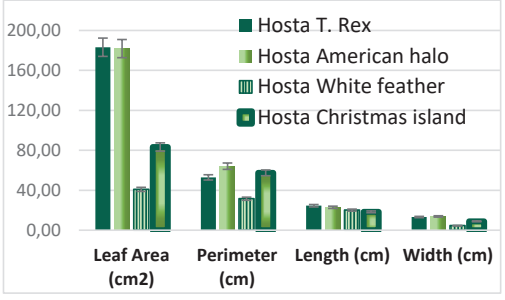

Figure 2. Biometrical measurements of the leaves (cm^2/cm)

As for the perimeter, the variant V_2 had the highest value (64.08 cm) and V_3 had the lowest value (31.51 cm), mainly correlated with leaf width.
The length of the leaves was between 24.50 cm for V_1 and 18.40 cm for V_4.
The width of the leaves was between 13.79 cm for V_2 variant and 4.59 cm for V_3.
The perimeter and the width showed the highest values in the V_2 variant and the smallest in the V_3 variant.
The determinations for the leaves of Hosta plants were made with the LCPro+ analyzer in a relatively small range of variation of the intensity of photosynthetic active radiation of 1518-1586 µmol m^{-2} s^{-1}, at temperature 36-38.6ºC (Table 2). In these conditions, it was registered different degrees of stomatal opening (between 0.06 - V2 and 0.22 - V3) that influenced physiological parameters.

Table 2. Intensity of photosynthesis, transpiration and respiration of Hosta leaves

Variant	Light intensity on leaf (Q leaf) µmol $m^{-2}s^{-1}$	Leaf Temp °C	Gs Degree of stomatal opening	Photosynthesis µmol CO_2 m^{-2} s^{-1}	Transpiration mmol H_2O m^{-2} sec^{-1}	Respiration µmol CO_2 m^{-2} s^{-1}
V_1	1586	36.5	0.15	19.85	4.53	3.17
V_2	1528	36.0	0.06	2.33	2.54	6.16
V_3	1518	38.3	0.12	21.43	4.51	2.81
V_4	1579	38.6	0.22	22.08	6.12	6.55

Q leaf - intensity of light incident on leaf, T°C - leaf temperature

The intensity of the photosynthesis process ranged from 2.33 µmol CO_2 m^{-2} s^{-1} for *American halo* (with green inside leaves) to 22.08 µmol CO_2 m^{-2} s^{-1} for *Christmas island* (with dark green on the edges). According to Zhang et al. (2018), the intensity of photosynthesis is higher in the green areas of the leaves compared to the yellow areas for the species *Aucuba japonica*.
The leaf surface and the position of the leaves against the solar radiation are important factors that condition the intensity of the photosynthesis process (Burzo et al., 2004). The results obtained by Toshoji et al. (2012), in the study over 12 ornamental plants, among which *Hosta* sp. *Reversed,* demonstrated that gross photosynthetic rates were lower in white sectors than in green sectors. Loss or reduction of green colour in the white sectors of the variegate leaves did not result from an optical effect (such as random reflection of light), simply was an effect of functional chloroplasts decrease.

Regarding the intensity of the transpiration process according to the data presented in Table 2, the variation limits were between 2.54 mmol H_2O m^{-2} sec^{-1} for *American halo* and 6.12 mmol H_2O m^{-2} sec^{-1} for *Christmas island*. The intensity of transpiration varies with the species, the age of the plant and the environmental conditions (Toma & Jităreanu, 2007).

A significant correlation between intensity of the photosynthesis and transpiration was recorded ($R^2 = 0.79$, $y = 5.73\ x - 8.94$, where y = intensity of photosynthesis and x = intensity of transpiration), probably according to the degree of stomatal opening.

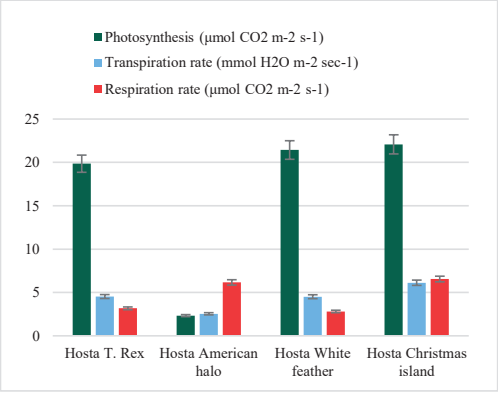

Figure 3. Intensity of the physiological processes

Regarding the intensity of the respiration process (Figure 3) it can be appreciate that the lowest value of the intensity of the respiration was recorded for V_3 (*White feather*), 2.81 µmol CO_2 m^{-2} s^{-1}, and the highest value was recorded for V_4 (*Christmas island*), 6.55 µmol CO_2 m^{-2} s^{-1}. According to Zhang et al. (2018), the respiration intensity in the yellow areas of the *Aucuba japonica* species is higher than photosynthesis. White sectors tended to exhibit dark respiration rates lower than those in green sectors (Toshoji et al., 2012).

Determinations regarding the content of chlorophyll and carotenoid pigments in the species of the *Hosta* genus led to the results presented in Table 3. They highlighted the modification of the content of assimilating pigments according to the species, as follows: the chlorophyll a content was higher in the case of *Christmas island*, the green sample (S_7), at a value of 19.82 mg/100 g, and the smallest quantity was recorded for the S_6 variant, *Christmas island*, the white sample, at the value of 0.4 mg/100 g, according to Figure 4.

The quantity of chlorophyll b ranged from 0.16 in sample S_6 (*Christmas island* white) to 14.56 in sample S_7 (*Christmas island* green).

It should be noted the increased content of chlorophyll and carotenoid pigments in S_7 (*Christmas island* green) in positive correlation with the photosynthesis rate.

Tabel 3. Assimilatory pigments content

Sample	Chl. a (mg/100 g fw)	Chl. b (mg/100 g fw)	Total chlorophyll (mg/100 g fw)	Carotenoids (mg/100 g fw)	Cl a/Cl b ratio
S1	18.93	7.74	26.67	5.41	2.45
S2	3.26	0.92	4.18	1.60	3.99
S3	18.61	7.27	25.88	5.67	2.56
S4	16.28	4.93	21.21	5.45	3.30
S5	17.08	5.73	22.81	5.95	2.98
S6	0.40	0.16	0.56	0.30	2.50
S7	19.82	14.6	34.38	6.24	1.36
S8	19.09	8.24	27.33	5.71	2.32

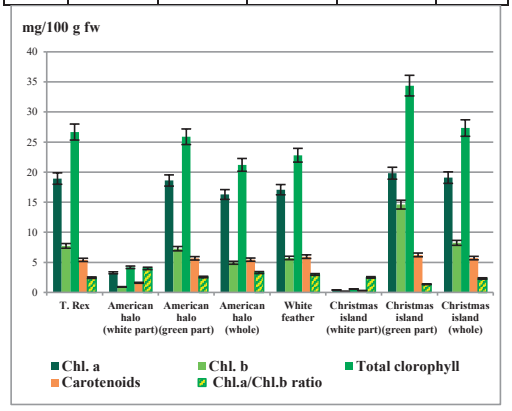

Figure 4. Content of chlorophyll and carotenoid pigments (mg/100g)

Following the analysis of the obtained results regarding the carotene content of the leaves, a smaller quantity of it was noticed in samples S_2 (1.6 mg/100 g) and S_6 (0.3 mg/100 g) compared to the other samples, where the quantity was over 5 mg/100 g.

The amount of chlorophyll a, chlorophyll b, total chlorophyll and carotene is higher in the

green areas of the leaves compared to the white areas.

The results are similar to those obtained by Zhang et al. (2018) for the *Aucuba japonica* species. In *Hosta "Gold Standard"* leaves, total Chls, Chl a, and Chl b in golden regions were obviously lower than in green regions of variegated leaves, and the Chl b accumulation was more reduced than Chl a (Yu et al., 2016).

The ratio between chlorophyll pigments and carotenoid pigments in the leaves may provide indications for leaf colour changes.

The content of chlorophyll pigments is correlated in the case of numerous plants with the intensity of the photosynthesis process and respectively with the accumulation of nutrients in the plants.

As for the dominant colour of the leaves determined with Winfolia software (Table 4, Figure 5), the colour recorded for the variant *Hosta T. Rex* was 100% green, the *Hosta American halo* variant had the dominant colour percentage of 78.93%, *Hosta White feather* of 92.95%, and in *Hosta Christmas island* the percentage of green from the total leaf area was 62.19%.

Tabel 4. Percentage of green color in the leaves

Sample	Dominant colour Green (%)	Secondary colour White (%)
Hosta *T. rex*	100.00	0.00
Hosta *American halo*	78.93	21.07
Hosta *White feather*	92.95	7.05
Hosta *Christmas island*	62.19	37.81

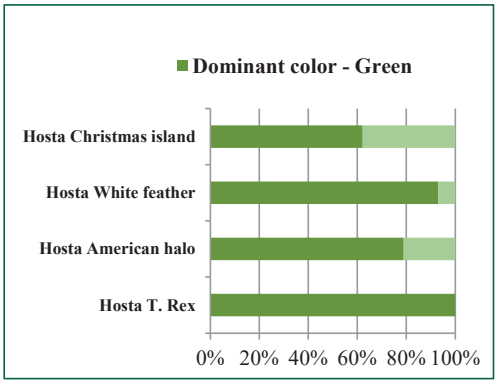

Figure 5. The dominant colour of the leaves

Leaf variegation is seen in many ornamental plants and is often caused by a cell-lineage type formation of white sectors lacking functional chloroplasts (Sakamoto et al., 2009).

CONCLUSIONS

The ornamental plants taken into study are useful resources for further research regarding the morphological aspects correlated with physiological processes that are carried out at the variegate leaf level. The results obtained varied according to the cultivars and the assimilatory pigments distribution in leaf, which positively influenced both the physiological processes in leaves and their dominant colour.

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