Volume 26(3), 83 - 88, 2022
JOURNAL of Horticulture, Forestry and Biotechnology
www.journal-hfb.usab-tm.ro

Variation in the content of photosynthetic pigments in the leaves of the species *Nandina domestica* Thunb.

Toța Cristina Elena[1], Szilagyi Claudia[1], Sala F.[2*]

Banat University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, 119 Aradului Street, 300645 Timișoara, Romania
[1]Horticulture Department, [2]Soil Science and Plant Nutrition Department

*Corresponding author. Email: florin_sala@usab-tm.ro

Abstract The study evaluated the content of photosynthetic pigments in the leaves of the species *Nandina domestica* Thunb. (Holy bamboo) the 'Obsessed' cultivar under the conditions of the autumn season. About 100 leaf samples were taken randomly from mature plants in the third decade of October. From the primary evaluation of the leaves, 10 categories of leaves (Lc) were selected according to the color of the leaves (intense green to anthocyanin colored leaves). Within each category, 5 leaves were analyzed and the chlorophyll content (Chl) and carotenoid content (Car) was determined. Based on the recorded chlorophyll (Chl) and carotenoid (Car) content values, the Chl:Car and Car:Chl ratios were calculated for each category of leaves. The chlorophyll content varied between 8.40 – 52.07±5.23 SPAD units, and the carotenoid content varied between 2.40 – 10.51±1.01 units. In the case of the calculated ratios, the recorded values varied between 3.00 – 4.95±0.21 in the case of the Chl:Car ratio, respectively between 0.20 – 0.33±0.01 in the case of the Car:Chl ratio. The single factor ANOVA test (Alpha=0.001) confirmed the statistical reliability of the data and the presence of the variance in the set of recorded experimental data (F>F crit). The variation of carotenoid content (Car) in relation to chlorophyll (Chl) was described by a polynomial equation of degree 2, under conditions of R^2=0.987, p<0.001, F=272. PCA correlation, facilitated obtaining a distribution diagram of the Leaf categories (Lc1 to Lc10) in relation to the determined photosynthetic pigments (Chl, Car) and the calculated ratios (Chl:Car, Car:Chl). According to PCA, PC1 explained 86.388% of variance, and PC2 explained 13.458% of variance. Cluster analysis facilitated obtaining the dendrogram based on Euclidean distances, in which the leaf categories (Lc) were associated based on similarity in relation to the evaluated physiological indices (Chl and Car), in conditions of statistical safety (Coph. Corr. = 0.908). Two clusters (C1 and C2) with several sub-clusters each resulted. From the analysis of the leaf categories (Lc) distribution in the dendrogram obtained and based on the calculated SDI values, the highest level of similarity was observed between Lc8 and Lc9 (SDI=0.6403), followed by Lc2 and Lc3 (SDI=0.8052).

Key words

Nandina domestica, Holy bamboo, ornamental plants, photosynthetic pigments, pigment ratio

The leaves faithfully express the state of vegetation of the plants [21; 3], and in the case of ornamental plants and especially ornamental plants through leaves, the leaves can have different shapes, sizes and colors on the same plant, in relation to the specifics of the genotype cultivated, ornamental quality indices and vegetation conditions [35; 29].

Different parameters and foliar indices were studied to evaluate the relationship of plants with vegetation factors [28], growth substrates [22], with the state of nutrition [6; 10], the water regime [26], stress factors [27], or with the attack of some diseases or pests [7]. Some studies have carried out different tests on the leaves of some plant species, as quality indicators of natural or anthropogenic ecosystems [8; 5; 20].

Different studies have highlighted that the photosynthetic pigments in the leaves vary according to the plant species [13; 16], latitude and longitude [15], seasons [18; 30], climatic conditions [12], technological factors [1], leaf age [2; 34], position in the crown / on the shoot [32], stress factors [23] as well as other conditions and influencing factors.

The photosynthetic pigments in the leaves of ornamental plants were studied in relation to the plant species (genotype), the general growing conditions [24; 33]. The chlorophyll content (SPAD) was used in different studies to characterize the relationship of plants with nutritional factors and growing conditions, or to differentiate some plant varieties [20; 4].

The coloring of the leaves of some plant species has been studied associated with the autumn

season, in relation to genetic, chemical and biochemical, physiological mechanisms that intervene in the synthesis of leaf pigments (chlorophyll, carotenoids and anthocyanins) [17; 25].

Nandina domestica Thunb. (Berberidaceae Family), considered in the present study, is a species native to Asia, from the Himalayas to Japan [19], and the name of the genus (*Nandina*) comes from the Japanese language (*nanten*) [11]. The plant grows as a shrub, with straight stems, pinnate, compound leaves. The initial leaves from the growth tips of the shoots have a pale pink color, they become green in the summer, and in the fall and winter they acquire a reddish-purple color. The species lends itself to different spaces, with an ornamental role through its leaves, but there have also been approaches from the perspective of invasive potential in certain areas [31].

The purpose of this study was to evaluate the leaves of the species *Nandina domestica* 'Obsessed' cultivar under the aspect of the content of photosynthetic pigments in the specific conditions of the autumn season.

Material and Method

The study analyzed the content of photosynthetic pigments in the leaves of the species *Nandina domestica* 'Obsessed' cultivar, under the conditions of the autumn season to capture the variation in the content of chlorophyll and carotenoids.

About 100 leaves were taken randomly from mature plants from the culture of ornamental plants (SC Atmosfera verde SRL) from Timisoara, Romania. Based on a primary evaluation, 10 leaf categories (Lc) were selected according to leaf color (intense green to anthocyanin colored leaves), figure 1.

Within each category, 5 leaves were analyzed and the chlorophyll content (Chl) and carotenoid content (Car) was determined. Chlorophyll content was determined using the Chlorophyllmeter SPAD 502Plus (KONICA MINOLTA). The carotenoid content was determined with the ACM-200 Plus device (OPTI-SCIENCES).

Lc1 Lc3 Lc5 Lc7 Lc10

Figure 1. Samples on different categories of leaves (Lc) in the species *Nandina domestica* 'Obsessed' cultivar (selective presentations)

Based on the recorded chlorophyll (Chl) and carotenoid (Car) content values, the Chl:Car and Car:Chl ratios were calculated for each category of leaves.

The obtained results were analyzed by appropriate statistical methods.

The statistical reliability of the results and the presence of variance in the experimental data series (ANOVA test), the degree of variability in the data series, the distribution of the leaf categories considered in the study in relation to determined parameters, as well as the level of similarity of Lc were evaluated. The processing and analysis of the recorded data was done in EXCEL and with the PAST software [14].

Results and Discussions

By analyzing the content of chlorophyll (Chl) and carotenoids (Car) in the leaves of the species *Nandina domestica* 'Obsessed' cultivar, the values of the chlorophyll pigments considered in the study were obtained and based on them the ratios Chl:Car and Car:Chl were calculated, table 1. The chlorophyll content varied between 8.40 – 52.07±5.23 SPAD units, and the carotenoid content (Car) varied between 2.40 – 10.51±1.01 units. In the case of the calculated ratios, the recorded values varied between 3.00 – 4.95±0.21 in the case of the Chl:Car ratio, respectively between 0.20 – 0.33±0.01 in the case of the Car:Chl ratio. In relation to the variation of the values of the two indices, Chl and Car, 10 categories of leaves (Lc1 to Lc10) were considered, table 1.

The graphic representation of the values per series of leaf categories is shown in figure 2, in the form of a normal probability plot, under conditions of correlation coefficient r>0.9 (r=0.947 for Chl; r=0.951 for Car; r=0.972 for Chl: Car; r=0.975 for Car:Chl).

The single factor ANOVA test led to the values presented in table 2, which confirms the presence of variance in the data set and the statistical reliability of the recorded data.

Table 1.
The values of the physiological indices (Chl, Car) and the ratios calculated for the leaf samples, species *Nandina domestica* 'Obsessed' cultivar

Trial	Chl	Car	Chl/Car	Car/Chl
Lc1	52.07	10.51	4.95	0.20
Lc2	45.30	9.90	4.58	0.22
Lc3	44.52	9.70	4.59	0.22
Lc4	40.20	9.50	4.23	0.24
Lc5	26.70	8.20	3.26	0.31
Lc6	17.70	5.90	3.00	0.33
Lc7	14.20	4.30	3.30	0.30
Lc8	12.60	3.60	3.50	0.29
Lc9	12.20	3.10	3.94	0.25
Lc10	8.40	2.40	3.50	0.29
SE	±5.23	±1.01	±0.21	±0.01

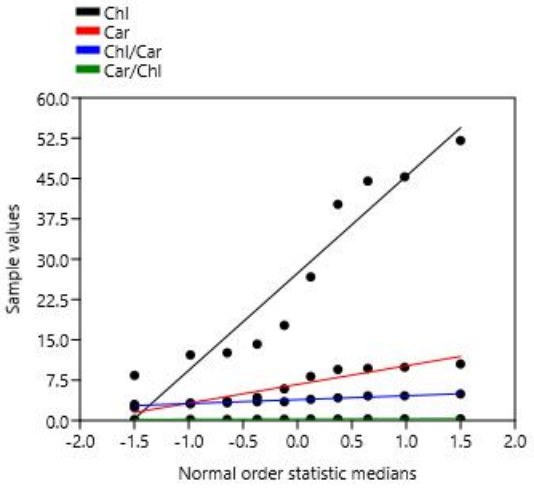

Figure 2. The graphic distribution in the form of a normal probability plot for the data series of leaf samples, the species *Nandina domestica* 'Obsessed' cultivar

Table 2.
ANOVA test for data series

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	4446.104	3	1482.035	20.83111	5.35E-08	4.377096
Within Groups	2561.23	36	71.14527			
Total	7007.333	39				

Alpha = 0.001

The variation of carotenoid content (Car) in relation to chlorophyll (Chl) was described by a polynomial equation of the 2nd degree, under conditions of R^2=0.987, p<0.001, F=272, with graphic representation in figure 3.

$$Car = -0.00483 \cdot Chl + 0.472 \cdot Chl - 1.419 \quad (1)$$

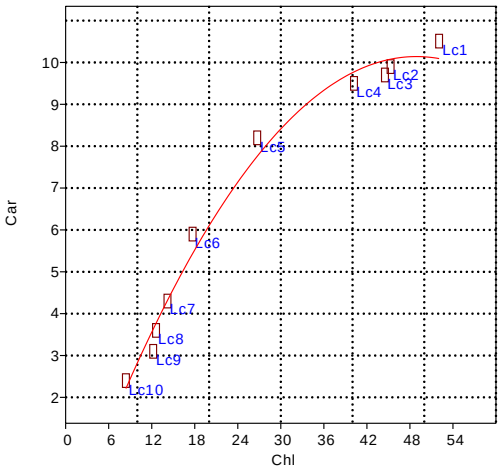

Figure 3. Distribution of Car in relation to Chl, leaves of *Nandina domestica* 'Obsessed' cultivar

PCA facilitated obtaining the diagram in figure 4 which includes the distribution of the studied leaf categories (Lc1 to Lc10) in relation to determined physiological indices (Chl, Car) and the calculated ratios (Chl:Car, Car:Chl). PC1 explained 86.388% of variance, and PC2 explained 13.458% of variance.

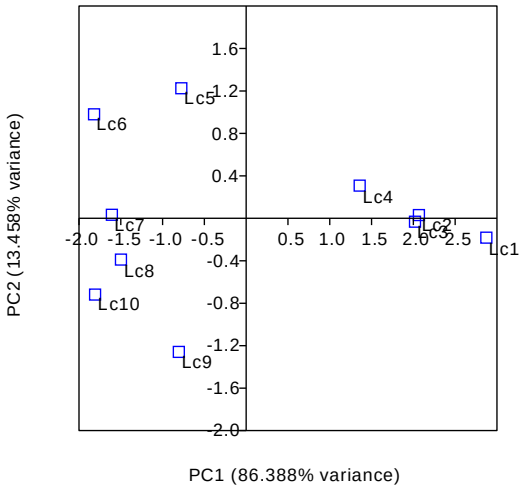

Figure 4. Distribution of leaf categories (Lc) in PCA diagram, *Nandina domestica* 'Obsessed' cultivar

Cluster analysis facilitated obtaining the dendrogram in figure 5, in which the leaf categories (Lc) were associated based on similarity in relation to the evaluated physiological indices (Chl and Car), in conditions of statistical safety (Coph. Corr.=0.908).

Two clusters (C1 and C2) with several sub-clusters each resulted. From the analysis of the distribution of Lc in the obtained dendrogram (figure 5) and based on the calculated SDI values, table 3, it resulted that the highest level of similarity was between Lc8 and Lc9 (SDI=0.6403), followed by Lc2 and Lc3 (SDI= 0.8052).

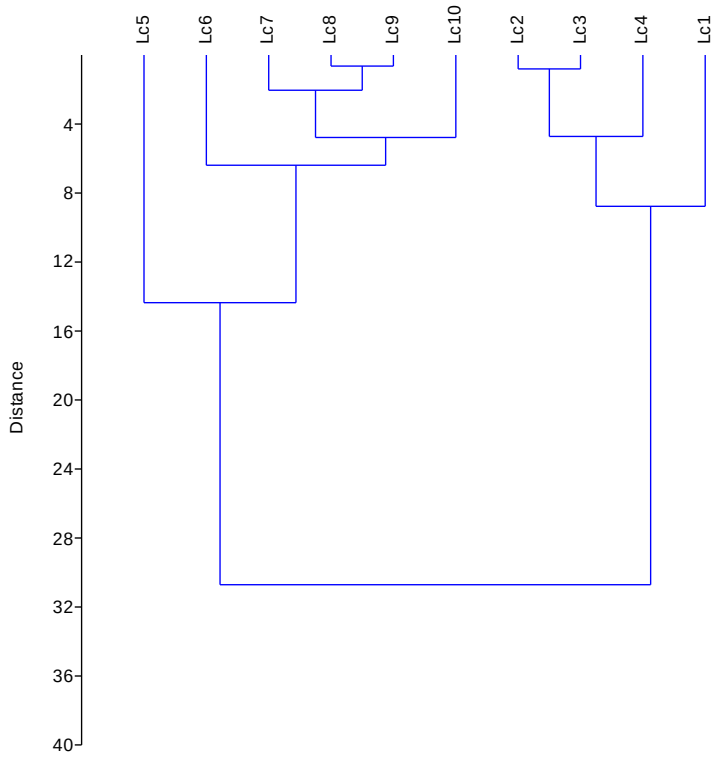

Figure 5. Dendrogram of leaf categories association in relation to Chl and Car, species *Nandina domestica* 'Obsessed' cultivar

Table 3

SDI values for leaf categories in relation to Chl and Car content, *Nandina domestica* species, 'Obsessed' cultivar

	Lc1	Lc2	Lc3	Lc4	Lc5	Lc6	Lc7	Lc8	Lc9	Lc10
Lc1		6.7974	7.5933	11.9130	25.4750	34.6780	38.3760	40.0700	40.5530	44.4170
Lc2	6.7974		0.8052	5.1157	18.6780	27.8880	31.6000	33.3010	33.7910	37.6540
Lc3	7.5933	0.8052		4.3246	17.8830	27.0880	30.7970	32.4980	32.9870	36.8500
Lc4	11.9130	5.1157	4.3246		13.5620	22.7860	26.5150	28.2240	28.7220	32.5830
Lc5	25.4750	18.6780	17.8830	13.5620		9.2892	13.0940	14.8310	15.3710	19.1970
Lc6	34.6780	27.8880	27.0880	22.7860	9.2892		3.8484	5.5946	6.1717	9.9368
Lc7	38.3760	31.6000	30.7970	26.5150	13.0940	3.8484		1.7464	2.3324	6.1033
Lc8	40.0700	33.3010	32.4980	28.2240	14.8310	5.5946	1.7464		0.6403	4.3681
Lc9	40.5530	33.7910	32.9870	28.7220	15.3710	6.1717	2.3324	0.6403		3.8639
Lc10	44.4170	37.6540	36.8500	32.5830	19.1970	9.9368	6.1033	4.3681	3.8639	

The variability evaluated based on the coefficient of variation (CV) was different in relation to the photosynthetic pigments and the calculated ratios. Thus, chlorophyll (Chl) showed much higher variability (CV=60.4346) compared to carotenoid pigments (Car) in the case of which the coefficient of variation had the value CV=47.4612. In the case of the calculated ratios, the coefficient of variation values were close, CV=17.2644 in the case of the Chl:Car ratio, respectively CV=16.8055 in the case of the Car:Chl ratio.

The obtained results contribute to the description of the behavior of the species *Nandina domestica* 'Obsessed' cultivar in the specific conditions of ornamental plant culture within SC Atmosfera verde SRL from Timisoara, Romania.

The data presented fall within the limits described in other researches, which addressed the respective species, and can be considered for other studies.

Conclusions

Based on the leaf samples collected for the study, 10 categories were differentiated according to color, from intense green to red-violet.

The analysis of the content of photosynthetic pigments (Chl, Car) led to obtaining a set of data, which presented a specific variant and statistical certainty (ANOVA test) in relation to the color category of the leaves.

The regression analysis led to a polynomial equation that described the variation of Car in relation to Chl under conditions of statistical safety.

The ratios between the photosynthetic pigments (Chl:Car and Car:Chl) expressed variable weights in relation to the e-content values recorded per leaf category (Lc).

The PCA analysis facilitated finding out the way in which the categories of plants (Lc) taken into account were distributed in relation to the parameters considered (Chl, Car, Chl:Car and Car:Chl). The cluster analysis facilitated finding the level of similarity that the categories of leaves presented, in relation to the content of photosynthetic pigments (Chl, Car) and ratios analyzed (Chl:Car, Car:Chl).

Acknowledgments

The authors would like to thank SC Atmosfera verde SRL, from Timisoara, Romania, for facilitating the conduct of this study through access to the biological material to collect the leaf samples.

References

[1] Abd-Elkader D.Y., Mohamed A.A., Feleafel M.N., Al-Huqail A.A., Salem M.Z.M., Ali H.M., Hassan H.S. 2022. Photosynthetic pigments and biochemical response of zucchini (*Cucurbita pepo* L.) to plant-derived extracts, microbial, and potassium silicate as biostimulants under greenhouse conditions. Frontiers in Plant Science 13: 879545.

[2] Bielczynski L.W., Łącki M.K., Hoefnagels I., Gambin A., Croce R. 2017. Leaf and plant age affects photosynthetic performance and photoprotective capacity. Plant Physiology 175(4): 1634-1648.

[3] Brendel O. 2021. The relationship between plant growth and water consumption: a history from the classical four elements to modern stable isotopes. Annals of Forest Science 78: 47.

[4] Constantinescu C., Herbei M., Rujescu C., Sala F. 2018. Model prediction of chlorophyll and fresh biomass in cereal grasses based on aerial images. AIP Conference Proceedings 1978(1): 390003.

[5] Datcu A.-D., Sala F., Ianovici N. 2017. Studies regarding some morphometric and biomass allocation parameters in the urban habitat on *Plantago major*. Research Journal of Agricultural Science 49(4): 96-102.

[6] Demotes-Mainard S., Boumaza R., Meyer S., Cerovic Z.G. 2008. Indicators of nitrogen status for ornamental woody plants based on optical measurements of leaf epidermal polyphenol and chlorophyll contents. Scientia Horticulturae 115: 377-385.

[7] Drienovsky R., Nicolin A.L., Rujescu C., Sala F. 2017. Scan Sick & Healthy Leaf – A software application for the determination of the degree of the leaves attack. Research Journal of Agricultural Science 49(4): 225-233.

[8] Fanelli G., Tescarollo P., Testi A. 2006. Ecological indicators applied to urban and suburban floras. Ecological Indicators 6(2): 444-457.

[9] Fort F., Freschet G.T. 2020. Plant ecological indicator values as predictors of fine-root trait variations. Journal of Ecology 108(4): 1565-1577.

[10] Ge S., Zhu Z., Peng L., Chen Q., Jiang Y. 2018. Soil nutrient status and leaf nutrient diagnosis in the main apple producing regions in China. Horticultural Plant Journal 4(3): 89-93.

[11] Gledhill D. 2008. The Names of Plants. Cambridge University Press. ISBN 9780521866453 (hardback), ISBN 9780521685535 (paperback). pp 145, 268.

[12] Gori A., Brunetti C., Dos Santos Nascimento L.B., Marino G., Guidi L., Ferrini F., Centritto M., Fini A., Tattini M. 2021. Photoprotective role of photosynthetic and non-photosynthetic pigments in *Phillyrea latifolia*: Is their "antioxidant" function prominent in leaves exposed to severe summer drought? International Journal of Molecular Sciences 22(15): 8303.

[13] Hallik L., Kazantsev T., Kuusk A., Galmés J., Tomás M., Niinemets Ü. 2017. Generality of relationships between leaf pigment contents and spectral vegetation indices in Mallorca (Spain). Regional Environmental Change 17: 2097-2109.

[14] Hammer Ø., Harper D.A.T., Ryan P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica, 4(1), 1-9.

[15] Kaluthota S., Pearce D.W., Evans L.M., Letts M.G., Whitham T.G., Rood S.B. 2015. Higher photosynthetic capacity from higher latitude: foliar characteristics and gas exchange of

southern, central and northern populations of *Populus angustifolia. Tree Physiology 35(9): 936-948.*
[16] Li Y., He N., Hou J., Xu L., Liu C., Zhang J., Wang Q., Zhang X., Wu X. 2018a. Factors influencing leaf chlorophyll content in natural forests at the biome scale. Frontiers in Ecology and Evolution 6: 64.
[17] Li Wx., Yang Sb., Lu Z., He Z., Ye Y., Zhao B., Wang L., Jin B. 2018b. Cytological, physiological, and transcriptomic analyses of golden leaf coloration in *Ginkgo biloba* L. Horticulture Research 5: 12.
[18] Liu C., Sun P.S., Liu S.R., Lu H.B., Chen Z.C., Liu X.J. 2017. Leaf photosynthetic pigment seasonal dynamic of *Quercus aliena* var. acuteserrata and its spectral reflectance response under throughfall elimination. Ying Yong Sheng Tai Xue Bao 28(4): 1077-1086.
[19] Onica E., Roșca I., Palanceanu A., Cutcovschi-Mușțiuc A., Mîțu V. 2020. The features of growth, development and cultivation of *Nandina domestica* Thunb. under the climatic conditions of the Republic of Moldova. Journal of Plant Development 27: 95-101.
[20] Pîrvulescu A., Sala F., Boldea M. 2015. Variation of chlorophyll content in sunflower under the influence of magnetic nanofluids. AIP Conference Proceedings 1648: 67009-1 – 67009-4.
[21] Poethig R.S. 2013. Vegetative phase change and shoot maturation in plants. Current Topics in Developmental Biology 105: 125-152.
[22] Sardoei A.S., Rahbarian P. 2014. Effect of different media on growth indexes of ornamental plants under system mist. European Journal of Experimental Biology 4(2): 361-365.
[23] Sherin G., Raj Aswathi K.P., Puthur J.T. 2022. Photosynthetic functions in plants subjected to stresses are positively influenced by priming. Plant Stress 4: 100079.
[24] Shymanska O., Vergun O., Rankhmetov J., Fishchenko V. 2017. The content of photosynthetic pigments in the leaves of *Galega officinalis* L. and *Galega orientalis* Lam. cultivars. Agrobiodiversity for improving nutrition, health and life quality, (1). Retrieved fromhttps://agrobiodiversity.uniag.sk/ scientificpapers/article/view/110
[25] Sun S., Zhang Q., Yu Y., Feng J., Liu C., Yang J. 2022. Leaf coloration in *Acer palmatum* is associated with a positive regulator ApMYB1 with potential for breeding color-leafed plants. Plants 11: 759.
[26] Toscano S., Ferrante A., Romano D. 2019. Response of Mediterranean ornamental plants to drought stress. Horticulturae 5: 6.
[27] Toscano S., Branca F., Romano D., Ferrante A. 2020. An evaluation of different parameters to screen ornamental shrubs for salt spray tolerance. Biology 9(9): 250.
[28] Villacrés J., Fuentes A., Reszka P., Cheein F.A. 2021. Retrieval of vegetation indices related to leaf water content from a single index: A case study of *Eucalyptus globulus* (Labill.) and *Pinus radiata* (D. Don.). Plants (Basel). 10(4): 697.
[29] Wang J., Shen J., Gu M., Wang J., Cheng T., Pan H., Zhang Q. 2017. Leaf coloration and photosynthetic characteristics of hybrids between *Forsythia* 'Courtaneur' and *Forsythia koreana* 'Suwon Gold'. HortScience 52(12): 1661-1667.
[30] Way D.A., Stinziano J.R., Berghoff H., Oren R. 2017. How well do growing season dynamics of photosynthetic capacity correlate with leaf biochemistry and climate fluctuations? Tree Physiology 37(7): 879-888.
[31] Wilson S.B., Rycyna J., Deng Z., Knox G. 2021. Summary of 26 heavenly bamboo selections evaluated for invasive potential in Florida. HortTechnology 31(4): 367-381.
[32] Zeng J., Ping W., Sanaeifar A., Xu X., Luo W., Sha J., Huang Z., Huang Y., Liu X., Zhan B., Zhang H., Li X. 2021. Quantitative visualization of photosynthetic pigments in tea leaves based on Raman spectroscopy and calibration model transfer. Plant Methods 17(1): 4.
[33] Zhang Q., Zhang M., Ding Y., Zhou P., Fang Y. 2018. Composition of photosynthetic pigments and photosynthetic characteristics in green and yellow sectors of the variegated *Aucuba japonica* 'Variegata' leaves. Flora 240: 25-33.
[34] Zhang J.-L., Li X.-G., Xu X.-H., Chen H.-P., Li Y.-L., Guy R.D. 2021. Leaf morphology, photosynthesis and pigments change with age and light regime in savin juniper. Plant Biology 23(6): 1097-1108.
[35] Zhao D., Tao J., 2015. Recent advances on the development and regulation of flower color in ornamental plants. Frontiers in Plant Science 6: 261.