MolBreed Publisher

Plant Gene and Trait 2022, Vol.13, No.3, 1-9
http://genbreedpublisher.com/index.php/pgt

Research Article Open Access

Leaf Anatomic Structure and Cold Tolerance Evaluation of 6 *Nymphaea* Species

Kuan Yang

Shanghai Chenshan Botanical Garden, Shanghai, 201602, China

Corresponding author email: yangkuan007001@163.com

Plant Gene and Trait, 2022, Vol.13, No.3 doi: 10.5376/pgt.2022.13.0003

Received: 08 Aug., 2022

Accepted: 12 Aug., 2022

Published: 20 Aug., 2022

Preferred citation for this article:

Yang K., 2022, Leaf anatomic structure and cold tolerance evaluation of 6 *Nymphaea* species, Plant Gene and Trait, 13(3): 1-9 (doi: 10.5376/pgt.2022.13.0003)

Abstract 10 leaf anatomical indexes of 6 *Nymphaea* species were observed and measured, and the cold tolerance was evaluated by cluster analysis and membership function. The result showed that *Nymphaea* is of bifacial leaf, stomata are only distributed in upper epidermis, and the thickness of upper epidermis was smaller than that of lower epidermis. Leaf thickness has significant differences and *Nymphaea potamophila* had the most compact leaf structure while *N.alba* had the most loose. Through cluster analysis, 10 indicators were divided into 3 categories, and indicators with the largest correlation coefficients were leaf thickness, stomatal density andpalisade tissue-spongy tissue ratio, which were found to be the main factors to evaluate the cold resistance of different *Nymphaea* species. The order of the six species based on cold tolerance from strong to weak is *N.alba*＞*N.potamophila*＞*N.lotus*＞*N.immutabilis*＞*N.colorata*＞*N.minuta*.

Keywords *Nymphaea*; Leaf; Anatomic structure; Cold tolerance

Waterlily (*Nymphaea L.*) is a perennial aquatic plant, with more than 50 native species and more than 1 000 cultivated varieties (Huang et al., 2008, China Forestry Press, pp.23-28; Li et al., 2019). According to its demand for survival temperature, it can be divided into two ecological types: cold tolerant *Nymphaea* species and tropical *Nymphaea* species. The former is mainly naturally distributed in subtropical and temperate regions, and the latter is mainly distributed in tropical regions. The flower colors of tropical *Nymphaea* species are more abundant than cold tolerant species, but cold tolerance is poor, and it is impossible to overwinter naturally in northern regions, which is the main limiting factor affecting the introduction and cultivation of tropical *Nymphaea* species in northern regions.

Temperature is an important factor affecting the growth of plants. Plants form an adaptive anatomic structure in the long-term adaptation to specific environmental conditions. In the evaluation of cold tolerance of plants, structural characteristics are also an important reference index. In which, as one of the main exposed organs of plants, the anatomical structure of leaves not only affects the physiological and biochemical functions of plants, but also reflects the adaptability of plants to environmental temperature. The results showed that the thickness of upper epidermis, the thickness of spongy tissue, the tissue structure tightness and the stomatal density of *Camellia oleifera* leaves were the main indicators affecting the cold resistance (Zeng et al., 2020), while the thickness of palisade tissue, the palisade tissue-spongy tissue ratio and the thickness of main vein were the main indicators affecting the cold resistance of Michelia plants (Li et al., 2017). As an important index, the morphological and anatomical characteristics of leaves have been applied in the evaluation of the cold resistance of *Hemerocallis fulva* (Di et al., 2019), *Caragana sinica* (Ma et al., 2020), *Cinnamomum camphora* (Wang et al., 2013) and other plants. The evaluation indexes are different among different plants, but in general, the closer the leaf structure is, the stronger the cold resistance is. At present, the research on *Nymphaea* mainly focuses on genetic diversity (Su et al., 2020), cold and shade tolerance of varieties (Ji, 2016; Liu et al., 2020), chemical component extraction (Huang et al., 2020), and other fields. The anatomy of the leaves of *Nymphaea* is mainly aimed at cold tolerant species (Chen et al., 1987; Hu et al., 2003), and the study on the anatomical structures of the other four subgenera of *Nymphaea* has not been reported. In this study, one kind of cold tolerant *Nymphaea* and five kinds of tropical

Nymphaea were taken as the research objects. The anatomical structure of their leaves was observed to evaluate the difference of their cold tolerant ability, so as to provide a basis for the promotion, application and new variety cultivation of *Nymphaea*.

1 Results and Analysis

1.1 Comparison of leaf thickness of different *Nymphaea*

The average thickness of the leaves of the six *Nymphaea* species is 427.90 μm (Table 1). There are significant differences among different species, among which *Nymphaea alba* has the thickest leaves, which is 803.66 μm. The leaves of *Nymphaea potamophila* are the thinnest (145.50 μm). Thickness is an important index to measure the cold resistance of plant leaves. The thicker the leaves, the higher the water content. Under low temperature conditions, it can delay the damage to the leaves. Among the six *Nymphaea* species, cold resistant *Nymphaea alba* have the thickest leaves, which are significantly higher than the other four subgenera. It is also consistent with the daily observation results.

1.2 Characteristics of leaf epidermis of different *Nymphaea*

The upper and lower epidermis of *Nymphaea* leaves are composed of monolayer cells, and the thickness of the lower epidermis is 12.55~22.35 μm. *N.minuta* is the highest, which is significantly higher than the other five species. The thickness of the upper epidermis of *Nymphaea* leaves is slightly lower than that of the lower epidermis, with a thickness of 7.64~16.79 μm. And the *N.immutabilis* is the highest, and the thickness of the upper and lower epidermis of the leaves of *N. potamophila* is lower than that of the other five *Nymphaea* species (Figure 1).

The stomata of different *Nymphaea* are long oval to round, which are only distributed on the upper epidermis of leaves. The average size of stomata is 130.08 μm^2, the average density is 548.35 /mm^2, and the coefficient of variation of stomatal density among different *Nymphaea* is 22.82%. The stomatal density of *N.alba* is the lowest (358.33 /mm^2). The stomatal density of two species of Subgen. *Brachyceras* Casp. is close, higher than that of the other four subgenera. The coefficient of variation of stomatal area among different *Nymphaea* was 22.47%. The stomatal area of *N. lotus* was the largest, which was 152.69 m^2, and that of *N.immutabilis* was the smallest of 80.27 μm^2 (Figure 2).

1.3 Structural characteristics of mesophyll in different *Nymphaea* species

The mesophyll structure of *Nymphaea* leaves is divided into palisade tissue and sponge tissue. Palisade tissue is composed of relatively closely arranged cylindrical cells, with a thickness of 33.74~145.30 μm. Compared with Michelia, *Cinnamomum camphora* and other plants, its structure is loose, which may be due to its participation in the ventilation process of upper epidermal pores. While the sponge tissue is composed of irregular cells, with a thickness of 99.91~625.16 μm (Figure 2). Among the six kinds of *Nymphaea*, the thickness of palisade tissue and sponge tissue of *N.alba* is the largest, and that of *N. potamophila* is the smallest, which is significantly different from other *Nymphaea*. *N. lotus* and *N. potamophila* have higher palisade tissue-spongy tissue ratio and tissue tightness, while *N.alba* have lower palisade tissue-spongy tissue ratio and tissue tightness than other *Nymphaea*, which are 0.26 and 18.64, respectively. The tissue structure looseness of the six *Nymphaea* was 63.05~77.78, in which *N.alba* was the highest, and *N. potamophila* was the lowest. In general, the leaf structure of *N. potamophila* is the most compact among the six *Nymphaea* species, while *N.alba* is relatively loose.

Table 1 Observation results of tissue structures in leaves of six species of *Nymphaea*

Nymphaea species	Leaf thickness /μm	Thickness of upper epidermis /μm	Thickness of palisade tissue /μm	Thickness of spongy /μm	Thickness of lower tissue epidermis/μm	Palisade tissue-spongy tissue ratio	Tissue structure tightness/%	Tissue structure looseness/%	Stomatal density/(mm^{-2})	Stomatal size /(μm^2)
Nymphaea alba	803.66a	16.20a	145.30a	625.16a	20.36ab	0.23b	18.09c	77.78a	358.33d	148.22a
Nymphaea immutabilis	600.27b	16.79a	119.33b	445.33b	20.59ab	0.27b	19.90bc	74.11b	577.50b	80.27c
Nymphaea lotus	270.19d	10.41b	63.11d	179.52d	15.67cd	0.35a	23.35a	66.44c	572.69b	152.69a
Nymphaea colorata	330.93d	14.70a	60.01d	239.89c	17.34bc	0.26b	18.70bc	71.56b	645.00ab	130.95ab
Nymphaea minuta	403.96c	14.78a	75.42c	289.59c	22.35a	0.26b	18.64bc	71.72b	683.33a	120.27b
Nymphaea potamophila	158.42e	7.64c	33.74e	99.91e	12.55d	0.34a	21.37ab	63.05c	453.26c	148.10a
Mean	427.90	13.42	82.82	313.23	18.14	0.29	20.01	70.78	548.35	130.08
Cv	52.15	27.80	47.30	58.19	24.57	19.30	13.12	7.72	22.82	22.47

Note: Different letters represent significant differences at 0.05 level

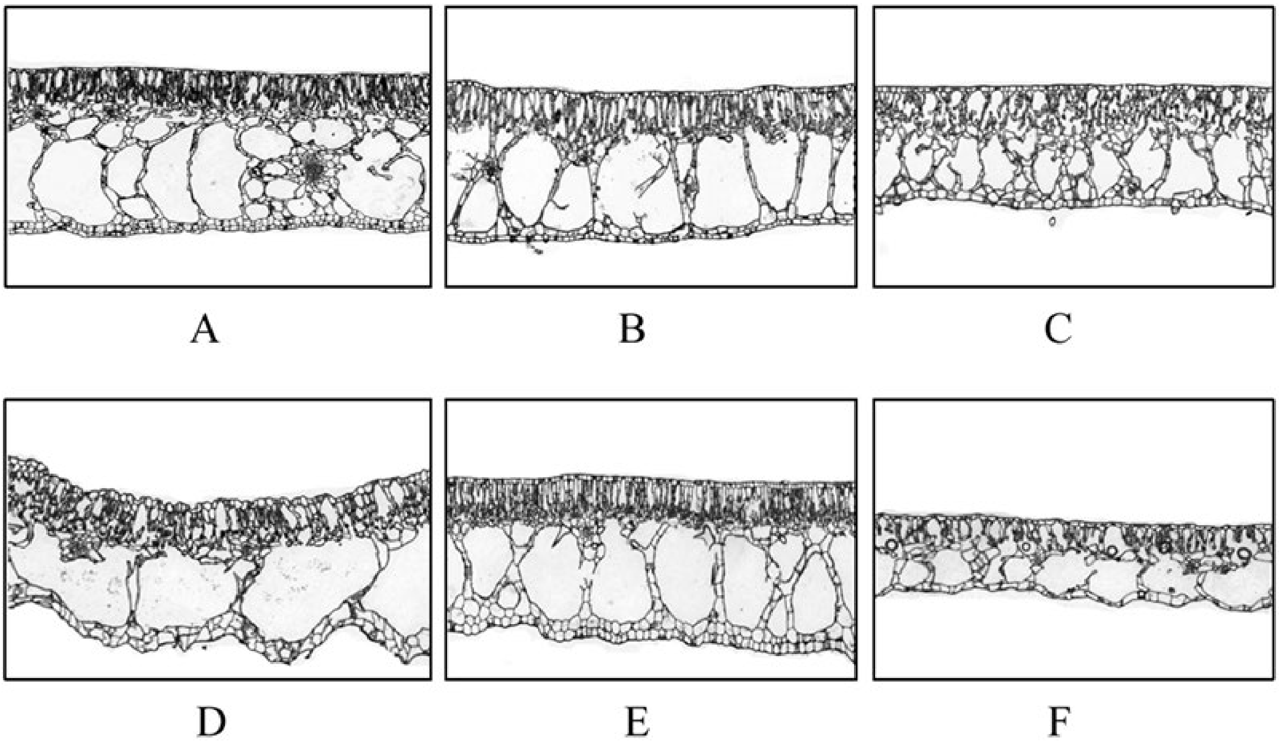

Figure 1 Leaf cross sections of six species of *Nymphaea*

Note: A *N.alba*, B *N.immutabilis,* C *N. lotus,* D *N.colorata,* E *N.minuta,* F *N. potamophila;* The scale in the figure is 100 μm

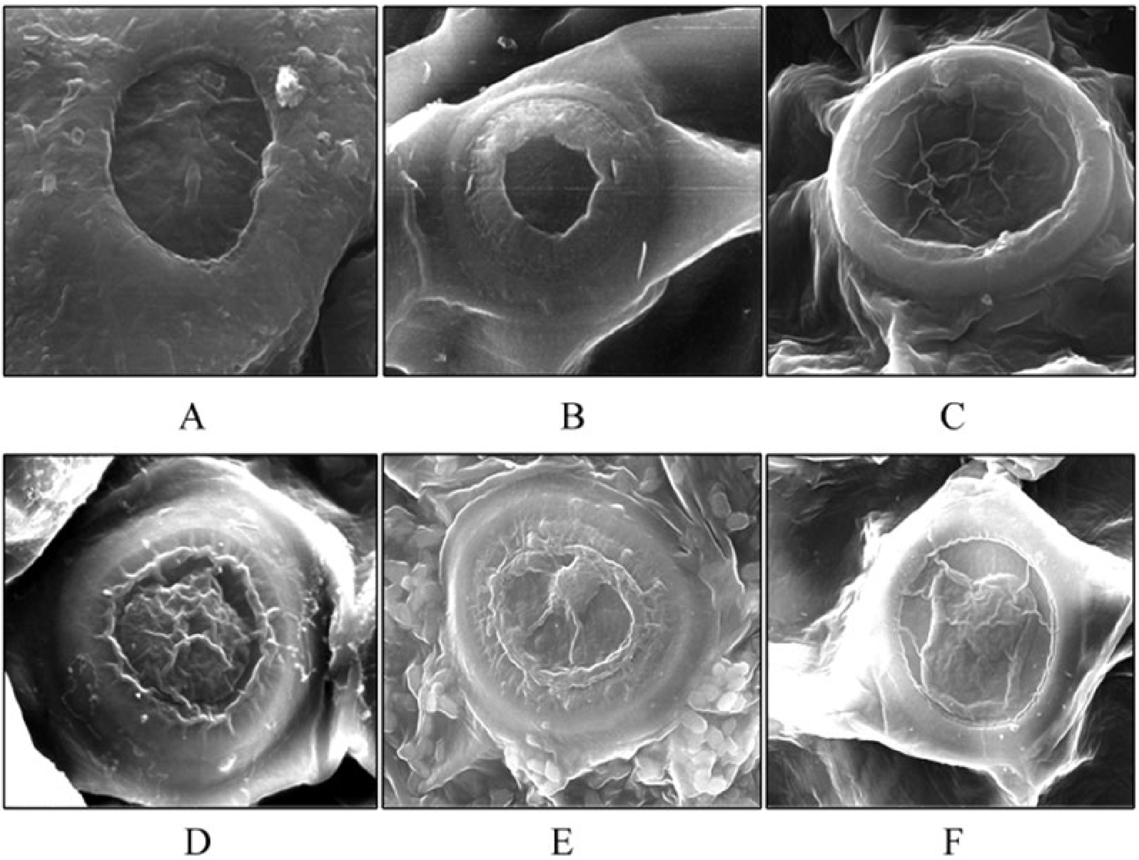

Figure 2 Lamina up-epidermis stoma of six species of *Nymphaea*

Note: A *N.alba*, B *N.immutabilis,* C *N. lotus,* D *N.colorata,* E *N.minuta,* F *N. potamophila;* The scale in the figure is10 μm

1.4 Comprehensive evaluation of cold resistance of different *Nymphaea* species

1.4.1 Cluster analysis and screening of cold resistance related indicators

After the standardization of 10 anatomical structure indexes of *Nymphaea* leaves, cluster analysis was carried out. The 10 indexes could be clustered into 3 groups. Group I included 5 indexes: total thickness of leaves, thickness of palisade tissue and sponge tissue, tissue looseness, and thickness of upper and lower epidermis; Group II only included stomatal density. Group III included the palisade tissue-spongy tissue ratio, tissue tightness and stomatal size (Figure 3).

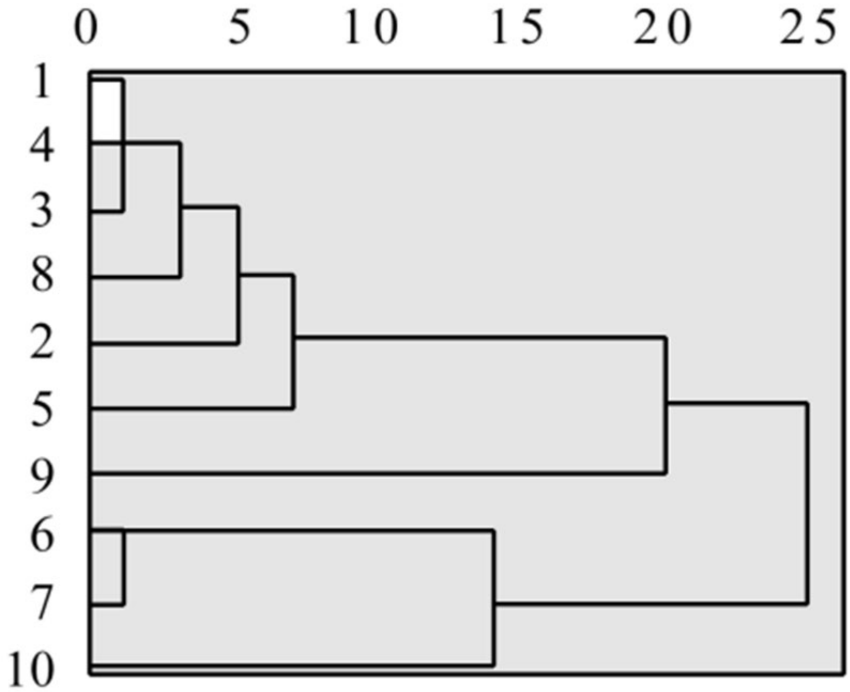

Figure 3 Cluster analysis result of ten leaf anatomic structure indexes

Note: 1: Total leaf thickness; 4: Spongy tissue; 3: Palisade tissue; 8: Tissue structure looseness; 2: Upper epidermis; 5: Lower epidermis; 9: Stomatal density; 6: Palisade tissue-spongy tissue ratio; 7: Tissue structure tightness; 10: Stomatal size

The correlation matrix of leaf structure indicators is shown (Table 2), and the typical indicators of the three types of indicators are determined according to the correlation index calculated by formula (5). The results showed (Table 3) that the typical indicators of the three types of indicators are total leaf thickness, stomatal density and palisade tissue-spongy tissue ratio.

1.4.2 Comprehensive evaluation results

According to formulas (6) and (7), calculate the membership function values of the typical indicators of each *Nymphaea*, and then obtain the average membership degree of its cold resistance. The larger the average membership degree is, the stronger the cold resistance of the *Nymphaea* leaves is. The results showed that the cold resistance of the six *Nymphaea* species leaves is as follows: *N.alba*>*N. potamophila*>*N. lotus*>*N.immutabilis*>*N.colorata*>*N.minuta* (Table 4).

Table 2 Correlative matrix of leaf structure indexes

Index	Leaf thickness	Thickness of upper epidermis	Thickness of palisade tissue	Thickness of spongy tissue	Thickness of lower epidermis	Palisade tissue-spongy tissue ratio	Tissue structure tightness	Tissue structure looseness	Stomatal density	Stomatal size
Leaf thickness	1.000	-	-	-	-	-	-	-	-	-
Thickness of upper epidermis	0.744**	1.000	-	-	-	-	-	-	-	-
Thickness of palisade tissue	0.974**	0.695**	1.000	-	-	-	-	-	-	-
Thickness of spongy tissue	0.998**	0.734**	0.964**	1.000	-	-	-	-	-	-
Thickness of lower epidermis	0.601**	0.657**	0.544**	0.588**	1.000	-	-	-	-	-
Palisade tissue-spongy tissue ratio	-0.684**	-0.743**	-.526**	-0.704**	-.634**	1.000	-	-	-	-
Tissue structure tightness	-0.494**	-0.619**	-0.300	-0.518**	-.544**	0.950**	1.000	-	-	-
Tissue structure looseness	0.881**	0.775**	0.803**	0.894**	0.627**	-0.870**	-0.680**	1.000	-	-
Stomatal density	-0.360	0.153	-0.370*	-0.377*	0.298	-0.014	-0.045	-0.120	1.000	-
Stomatal size	-0.286	-0.466**	-0.314	-0.263	-0.482**	0.269	0.179	-0.307	-0.427*	1

Note: * indicates a significant correlation at 0.05 level, and ** indicates a significant correlation at 0.01 level

Table 3 Correlation indexes and ranking of leaf structure indexes

Category	Index	Correlation index	Order in category
1	Leaf thickness	0.727	1
1	Thickness of palisade tissue	0.660	4
1	Thickness of spongy tissue	0.722	2
1	Tissue structure looseness	0.643	3
1	Thickness of upper epidermis	0.522	5
1	Thickness of lower epidermis	0.346	6
2	Stomatal density	1.000	1
3	Palisade tissue-spongy tissue ratio	0.487	1
3	Tissue structure tightness	0.467	2
3	Stomatal size	0.052	3

Table 4 Comprehensive evaluation of cold tolerance of six species of Nymphaea

Species	Leaf thickness	Stomatal density	Palisade tissue-spongy tissue ratio	Comprehensive evaluation	Order of cold tolerance
N.alba	1.000	1.000	0.000	0.667	1
N.immutabilis	0.685	0.326	0.364	0.458	4
N. lotus	0.173	0.340	1.091	0.535	3
N.colorata	0.267	0.118	0.273	0.219	5
N.minuta	0.381	0.000	0.273	0.218	6
N. potamophila	0.000	0.708	1.000	0.569	2

2 Discussion

As the main channel for gas exchange and transpiration of plant leaves, the differences in stomatal size and density can reflect the strength of plants' adaptability to adversity (Hee-Myong et al., 2001). It is generally believed (Fei et al., 1999; Wang et al., 2016) that plants with small stomatal density and stomatal size have relatively low respiratory efficiency and less water loss, so they have strong cold resistance. However, some studies (Wang et al., 2013; Zeng et al., 2020) have shown that there is little correlation between stomatal density, stomatal size and cold resistance. In this study, the high stomatal density of the tropical waterlily may be due to its distribution in the tropics, which is more conducive to transpiration and heat dissipation in the summer with high temperature and high light, while the cold resistant *Nymphaea alba* is on the contrary. The actual observation shows that the tropical waterlily has less leaf burns in summer, but the leaves are more prone to frostbite in late autumn. Overall, a small stomatal density may be more conducive to the cold resistance.

Many studies have shown that mesophyll tissue is closely related to the cold tolerance of leaves, but the results of different plants are different. Experiments showed that rose and camphor trees with strong cold resistance have thick palisade tissue, while sponge tissue is relatively thin. However, some studies have shown that the increase of palisade tissue, spongy tissue and leaf thickness is the result of plant adaptation to low temperature environment (Cai and Song, 2001). The results of this experiment showed that the thickness of leaves, palisade tissue and sponge tissue of *Nymphaea alba* were significantly higher than that of tropical *Nymphaea*, which was consistent with the evaluation results of cold tolerance. However, the palisade tissue-spongy tissue ratio of *Nymphaea alba* leaves is the lowest among all *Nymphaea* species, which is contrary to its evaluation results. At the same time, the anatomical results of other tropical *Nymphaea* species leaves are not completely consistent with their cold tolerance ranking, indicating that when evaluating the cold tolerance of *Nymphaea*, multiple indicators should be considered comprehensively, and one index should not prevail.

In this study, 10 indexes of leaf anatomical structure were selected to measure the five subgenera *Nymphaea*. Using cluster analysis method, three indexes of total leaf thickness, stomatal density and palisade tissue-spongy tissue ratio were selected to evaluate the cold resistance of *Nymphaea*. The results showed that *Nymphaea alba* had the strongest cold resistance, followed by *Nymphaea potamophila*, *Nymphaea lotus* and *Nymphaea immutabilis* in tropical waterlilies. Among the Subgen. *Brachyceras* Casp., *Nymphaea colorata* and *Nymphaea minuta* have the lowest structural cold tolerance. Subsequent studies can further determine the physiological indicators of *Nymphaea* leaves under low temperature stress, and then obtain more accurate results.

3 Materials and Methods

3.1 Experimental materials

The tested waterlily (*Nymphaea* L.) was taken from the waterlily Resource Garden of Shanghai Chenshan Botanical Garden, including *Nymphaea lotus*, *Nymphaea immutabilis*, *Nymphaea colorata*, *Nymphaea minuta*, *Nymphaea potamophila* and *Nymphaea alba*. Three plants of each species with similar growth potential were selected as the research objects, and fresh and mature leaves of *Nymphaea* were taken for standby in May 2020 (Table 5).

Table 5 Experimental materials

Species	Classification
Nymphaea alba	Subgen. *Castalia* Salisb.
Nymphaea immutabilis	Subgen. *Anecphya* Casp.
Nymphaea lotus	Subgen. *Lotos* Decandolle
Nymphaea colorata	Subgen. *Brachyceras* Casp.
Nymphaea minuta	Subgen. *Brachyceras* Casp.
Nymphaea potamophila	Subgen. *Hydrocallis* Casp.

3.2 Experimental methods

3.2.1 Stomatal observation

Take the parts on both sides of the main vein of waterlily leaves, cut the leaves into small squares of 5 mm, fix them with glutaraldehyde, wash them with PBS buffer, dehydrate them with ethanol gradient, and then replace them with isoamyl acetate. After drying and spraying gold, observe and take photos under the scanning electron microscope. Select 10 visual fields for each treatment, take the average value, and calculate the stomatal density. Photoshop was used to measure and calculate the stomatal size and coefficient of variation, where the stomatal size= π×stomatal length × stomatal width/4 (Gong et al., 2010).

3.2.2 Leaves structure observation

Took the central area of mature *Nymphaea* leaves, cut a 1 cm length segment away from the main vein, fixed, embedded and sliced it, and dyed it with safranine and solid green. Observed it with a microscope, and took photos with five fields of vision for each slice. Photoshop software was used to measure the thickness of leaves, upper epidermis, thickness of palisade tissue, thickness of spongy tissue and lower epidermis.

According to the method of Ma et al. (2020), calculated the palisade tissue-spongy tissue ratio, tissue structure tightness, tissue structure looseness and coefficient of variation of *Nymphaea* leaves respectively:

(1) Palisade tissue-spongy tissue ratio of leaves=Thickness of palisade tissue/Thickness of spongy tissue
(2) Tissue structure tightness=Thickness of palisade tissue/Thickness of leaves×100
(3) Tissue structure looseness=Thickness of spongy tissue/Thickness of leaves×100
(4) Coefficient of variation=Standard deviation/Mean×100%

3.2.3 Comprehensive evaluation of cold resistance

After the standardization of anatomical structure indexes of *Nymphaea* leaves, cluster analysis was carried out, and the relevant indexes of each index were calculated according to the method of Zeng et al. (2020): (5)$T=\Sigma r^2/(n-1)$, where: T is the correlation index of each anatomical index in their respective classification, n is the number of indicators in each category, and r is the correlation coefficient between each index and the indicators of the same group.

The membership function method was used to evaluate the cold resistance of different *Nymphaea* leaves. The index that is positively correlated with the cold resistance was calculated as follows: (6)$Y(X_i)=(X_i-X_{min})/(X_{max}-X_{min})$

The index calculation formula negatively related to cold resistance was as follows: (7)$Y(X_i)=1-(X_i-X_{min})/(X_{max}-X_{min})$, where: $Y(X_i)$ represents the subordinate value of cold tolerance of *Nymphaea*, X_i represents the score value of the i^{th} factor, X_{max} is the maximum score value of the i^{th} factor, and X_{min} is the minimum score value of the i^{th} factor.

Authors' Contribution

YK is the executor of this research design and experiment, completed data analysis and paper writing. The author read and approved the final manuscript.

Acknowledgements

This study was supported by the Science and Technology Project of Shanghai Landscaping & City Appearance Administrative Bureau (G182424; G192406).

References

Cai Y.L., and Song Y.C.,2001, Adaptive ecology of Lianas in Tiantong Evergreen Broad-Leaved Forest, Zhejiang, China I. Leaf Anatomical Characters, Zhiwu Shengtai Xuebao (Acta Phytoecologica Sinica), 25(1): 90-98

Chen S.P., Zhang S.M., and Lang J.H., 1987, The anatomy of leaf in Nymphaea Alba L, Nanjing Shida Xuebao (Journal of Nanjing Normal University(Natural Science)), (4): 69-75

Di Z.X., Su D.S., Hu M.J.L.T., Wu X.H., Zheng C.M., and Wei C.G., 2019, Cold resistance comparison of 11 varieties of *Hemerocallis fulva*, Xibu Linye Kexue (Journal of West China Forestry Science), 48(4): 137-141

Fei S.L., Fang J.Y., Fan Y.J., Zhao K., Liu X.J., and Cui K.M., 1999, Anatomical characteristics of leaves and woods of Fagus lucida and their relationship to ecological factors in Mountain Fanjingshan, Guizhou, China, Zhiwu Xuebao (Acta Botanica Sinica), 41(9): 1002-1009

Gong W., Peng J.H., Tian Q.Y., Yu W.T., Qiu B., and Zou M., 2010, Investigation of stomatal characteristics of six varieties of Hydrangea macrophylla, Beifang Yuanyi (Northern Horticulture), (13): 66-68

Hee-Myong R., Pan-Gi K., In-Bog L., Myoung-Soon Y., and Su-Young W., 2001, Photosynthetic characteristics and growth responses of dwarf apple (*Malus domestica* Borkh. Cv. Fuji) saplings after 3 years of exposure to elevated atmospheric carbon dioxide concentration and temperature, Trees, 15: 195-203 https://doi.org/10.1007/s004680100099

Hu G.W., Liu K.M., and Lei L.G., 2003, Comparative study on Peduncular Anatomy of three genera in Nymphaeaceae (s.l.) and Nymphoides peltatum of gentianaceae, Hunan Shifan Daxue Ziran Kexue Xuebao (J. Nat. Sci. Hunan Norm. Univ.), 26(4): 70-75

Huang Q.W., Mao L.Y., Long L.Y., and Tang Y.W., 2020, Optimization of essential oil from tropical water lily extracted by supercritical CO_2 and volatile components of essential oil analysis by GC-MS, Shipin Yanjiu yu Kaifa (Food Research and Development), 41(7): 188-195

Ji Q., 2016, Effect of low temperature stress on the cold resistance physiology of different varieties of tropical waterlily, Shanghai Nongye Xuebao (Acta Agriculturae Shanghai), 32(5): 114-118

Li R.X., Jin X.L., Hu X.J., Chai G.X., Cai M.Y., Luo F., and Zhang F.J., 2017, Analysis and comprehensive evaluation on cold resistance of six varieties of Michelia, Yingyong Shengtai Xuebao (Chinese Journal of Applied Ecology), 28(5): 1464-1472

Li S.J.,Wei Q., Chen C., Zhang Y., Wu Y.P., and Yu G., 2019, Breeding progress of waterlilies in China, Zhiwu Yichuan Ziyuan Xuebao (Journal of Plant Genetic Resources), 20(4): 829-835

Liu G.Y., Zhou W., Chen L., Wang H., Jin Q.J., Wang Y.J., Li N., and Xu Y.C., 2020, Comprehensive evaluation of shade tolerance of 11 Nymphaea tetragona cultivars, Zhiwu Ziyuan yu Huanjing Xuebao (Journal of Plant Resources and Environment), 29(1): 44-51

Ma H.Y., Lv X.X., Ji Y.N., and Li X.W., 2020, Leaf anatomical structure and drought resistance of 17 caragana species, Shuitu Baochi Yanjiu (Research of Soil and Water Conservation), 27(1): 340-347

Su Q., Yang Y.H., Tian M., Bo C.Y., Mao L.Y., Zhang J.Z., Pan J.C., and Lu J.S., 2020, Genetic diversity analysis and DNA fingerprinting Construction of waterlily germplasm resources, Redai Zuowu Xuebao (Chinese Journal of Tropical Crops), 41(2): 258-266

Wang N., Yuan M.L., and Su J.L., 2013, Relationship between leaf structure and cold resistance of several camphor tree species, Xibei Linxueyuan Xuebao (Journal of Northwest Forestry University), 28(4): 43-49

Wang R.L., Yu G.R., He N.P., Wang Q.F., Xu N., and Xu Z.W., 2016, Altitudinal variation in the covariation of stomatal traits with leaf functional traits in Changbai mountain, Shengtai Xuebao (Acta Ecologica Sinica), 36(8): 2175- 2184 https://doi.org/10.5846/stxb201411042162

Zeng J.L., Deng Q.E., Li J.A., Cheng J.Y., Deng X.Z., and Jiang D.Z., 2020, Relationship between leaf anatomic structure and cold tolerance in six Camellia oleifera cultivars, Jingjilin Yanjiu (Non-wood Forest Research), 38(1): 117-12