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STUDY ON THE ANATOMICAL MORPHOLOGY OF LOTUS VARIETIES *(Nelumbo nucifera* Gaertn.*)* IN VIETNAM

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ABSTRACT

This paper presents the anatomy of six lotus varieties collected at different lotus planting areas in Vietnam. The six different lotus varieties include GiaLong pink, PhuMong pink, VinhThanh red, High-yield, Concave white and Convex white lotus. The results indicate that the adaptation of these lotuses to the aquatic environment is presented by the morphological and anatomical characteristics of the roots, stem, leaves as well as the presence of air canals in the lotuses. In addition to the similarities in the lotuses composition, there are other differences. These include the number and size of woody vessels, air space characteristics the thickness of the epidermis, the size of the cortex and pericycle root, and the size of the anabolic tissues among the six lotus varieties. Our findings show that although the six lotus varieties are cultivated under the same natural conditions, they are highly diversified in terms of the internal anatomical morphology. This is an important feature in identifying the native lotus, which contributes to strengthening and maintaining the local brand name - the Hue lotus. Furthermore, the results obtained in this study allow for extending the anatomical and morphological descriptions of these studied species and to reveal their important diagnostic characters.

Keywords: Adaption; anatomy; morphology; lotus; ThuaThien hue province; Vietnam.

INTRODUCTION

The lotus *(Nelumbo nucifera* Gaertn.) belongs to the Nelumbonaceae [1], and is a perennial aquatic plant species [2]. It is rich in germplasm and more than 2000 cultivars have been cultivated through hybridization and natural selection [3]. There are two types in the world, including the *N. nucifera* Gaertn species, which are distributed throughout Asia and Oceania and the *N. lutea* Willd or American lotus, which is found in North and South America [4]. *Nelumbo nucifera*, also known as sacred lotus, has primarily been used as food throughout the Asian continent, and its medicinal values have been described in Ayurvedic and Traditional Chinese Medicine. The potential use of neferine, liensinine, isoliensinine, and nuciferine in clinical trials has been described by Sharma et al. [5]. In depth, mechanism of the potential chemical entities from *N. nucifera* via structure activity relationship has been explored to guarantee the stability and safety for the clinical

use. The lotus originated in Asia, specifically India, and then became distributed to China, Japan, Northeastern Australia and many other countries [6; Oro et al., 2013]. The lotus is consumed largely in Asia [7]. There is only one lotus species in Vietnam, the *N. nucifera,* which is mainly cultivated for its seeds. This species is widely distributed from the north to the south of the country and mainly grown in ponds, lakes and fields [8]. The lotus provides people with many benefits, from beautiful flowers, for food [9] and also medicinal herbs [10]. All parts of lotus including leaves, buds, petals, seeds can be used as ingredients for nutritious dishes or medical herbs. The latter provide curative properties for serious illnesses such as cancer, depression, obesity, diarrhea, cardiovascular disease, hypertension and insomnia [11]. Moreover, the lotus contains significant properties of alkaloids [12,13], flavonoids [14], steroids, triterpenoid, glycoside and polyphenol [15], the last being a powerful antioxidant useful in improving health systems of human beings [16]. Other parts of the lotus, such as the leaves, the seeds and embryo are found to be an important sources for theiranti-bacterial, anti-inflammatory and anti-viral properties [17,18]. In particular, the lotus flowers are often used in many festivals in Asian countries [19], symbolizing purity, holiness and immortality for many cultures for centuries [20,21]. In Hue, the lotus is associated with several famous sights, such as the Imperial City, the King's tombs, pagodas and temples. The lotus contributes to the unique beauty of Hue's architecture by adding a soft and graceful touch to these places. The value of the lotus is even more significant to Hue's inner city, where the Imperial City, a UNESCO World Heritage site, is located. In this area, the lotusis recognized for more than just its materialistic value. Indeed, it is the very symbol that represents the spirit of Buddhism, and as such it is employed to set the tone of worship in the most solemn and sacred of places. Comprehensive metabolomic profiling of these metabolites is of key importance to help understand their biological activities, and other chemical biology features. The study of Zhu et al. [22] provide an update on the current technological platforms, and workflow associated with metabolomic studies on lotus seeds, as well as insights into the application of metabolomics for the improvement of food safety and quality, assisting breeding, and promotion of the study of metabolism and pharmacokinetics of lotus seeds.

There are six different lotus varieties including the GiaLong pink lotus, the PhuMong pink lotus, the VinhThanh red lotus (also called the light red or dark pink lotus), the High-yield lotus, the Concave white lotus, and the Convex white lotus. All of these varieties are currently planted in ThuaThien Hue province. These lotus varieties are generally called pink and white lotus, with the High-yield lotus that is noted for its seeds originating in Dong Thap. Other lotus varieties are local species, which are famous because of their glamorous beauty, special taste and quality of tubers and seeds. These local lotus varieties are very popular with tourists, especially the "Hue Lotus" variety. In reality, the Hue lotus is often grown with other lotus varieties, notably the Hue pink lotus, which is often mixed up with the Dong Thap pink lotus and other unknown pink lotus varieties. This often leads to confusion between lotus varieties, making it difficult to distinguish among these various varieties and their lotus products. The classification of *N. nucifera* cultivars in ThuaThien Hue Province, Vietnam, such as the Pink and White lotus vartieties with their presumably unique anatomical characteristics, has not been studied to date. Hence, it is necessary to study the internal structure of lotus varieties to evaluate the diversity of their anatomical characteristics and their adaptability to the growing environment of the six lotus planted in ThuaThien Hue. This research provides important evidences for researchers, agronomists and policymakers in making plans to restore, conserve, exploit and develop local lotus varieties, which contribute great economic potential for ThuaThien Hue Province.

MATERIALS AND METHODS

Plant Materials

The six lotus varieties include GiaLong pink lotus, PhuMong pink lotus, VinhThanh red lotus, High-yield lotus, Concave white lotus and Convex white lotus (Fig. 1), all of which are cultivated in a number of areas in ThuaThien Hue Province, including Hue city, PhuLoc, PhongDien, PhuVang, HuongThuy and HuongTra districts.

Fig. 1. Flower morphology of the six lotus varieties cultivated in Vietnam

Methods

Anatomical structures of lotus organs were studied by cross sections. Samples were taken from around the leaf midribs measuring 5 mm, also from the middle region of the petiole measuring 2-5 mm, and lastly from the basal part of the root. These were excised and immediately fixed in formalin-acetic acid-alcohol (FAA) and stored at 4°C until sectioning. After preparation of the free hand sections, samples were washed with distilled water and placed in 5% sodium hypochlorite solution for 20 min for clearing and rinsed with distilled water. Following washing in 1% acetic acid for 2 min, the sections were then stained with methyl green (1/1000-1/10,000 in distilled water) for 10 seconds, then rinsed with distilled water. Continuing with the staining process, using carmine for 20 - 30 minutes, the sections were then rinsed with distilled water. The sections were ultimately embedded and mounted in sterile distilled water. Thin cut sections were observed under a microscope fitted with a digital camera and an ocular micrometer [23]. The size of components of rhizomes, stem and leaf were measured in micrometers (µm) [24].

Statistical Analysis

The variables and its interaction were analysed by ANOVA using STATGRAPHICS Plus for Windows. Means were separated by Duncan's multiple range test, $P \leq 0.05$.

RESULTS AND DISCUSSION

Anatomical Structure of Root

The epidermal root consists of two cell layers with the thickness of 97.50 - 124.09 µm on average and accounts for 5.91 - 8.29% the root radius. Among the six lotus varieties, the PhuMong pink lotus has the largest epidermis (124.0 µm) and the Convex white lotus has the smallest epidermis (97.5 µm) (Table 1).

The parenchymal cells have a spongy cortex root, covering the pericycle. The prenchyma cells are arranged into many inter cellular air spaces, allowing the roots to transport air and to breathe easily in the anaerobic environment. The innermost part of the cortex is the shell with thick and round cells allocated in consecutive rings to help improving the resistance ability of the root (Fig. 2 and Fig. 3). The cortex root size of the PhuMong pink lotus, the GiaLong pink lotus and the concave white lotus is quite large, ranging between 1256.4 and 1392.7 µm, accounting for 74.92 - 77.29% of the cortex radius. The other three lotus varieties, including the High-yield lotus, the VinhThanh red lotus and the convex

white lotus have smaller cortex roots, with 836.4 - 921.8 µm, accounting for 67.45 - 72.02% of the cortex radius. This difference is statistically significant (Table 1).

There are Phloem and Xylem cells in the pericycle which is interlaced, forming radial vascular bundles (Fig. 4). The pericycle size of the six lotus varieties are quite homogenous, ranging in 242.3 - 309.0 µm, accounting for 16.80 - 24.41% of the root radius. The GiaLong pink lotus has the largest pericycle size with 290.0 µm, while the convex while lotus has the smallest pericycle size with 242.3µm, accounting for 20.6% of the root radius (Table 1).

Table 1. Size of main structural compositions of lotus roots (µm)

Lotus variety	Epidermis		Cortex		Pericycle			
	Mean	Radius (%)	Mean	Radius (%)	Mean	Radius (%)	Number of wood vessels	Diameter of wood vessels
GiaLong pink lotus	117.5^{a}	6.82^{c}	1314.5^{b}	76.34^{b}	290^{d}	16.84^{c}	20.6^{a}	12.43^{b}
PhuMong pink lotus	124.0^{a}	7.39^{b}	1256.4^{c}	74.92^{c}	296.5^{c}	17.69^{d}	19.1^{b}	13.61^{a}
High-yield lotus	101.0^{bc}	7.90^{a}	921.8^{d}	72.06^{d}	256^{e}	20.03^{c}	20.3^{a}	10.34^{c}
VinhThanh red lotus	103.0^{bc}	8.14^{a}	854.5^{e}	67.45^{f}	309^{a}	24.41^{a}	20.7^{a}	12.63^{ab}
Convex white lotus	97.50^{c}	8.29^{a}	836.4^{e}	71.11^{e}	242.3^{f}	20.60^{b}	19.1^{b}	10.69^{c}
Concave white lotus	106.50^{b}	5.91^{d}	1392.7^{a}	77.29^{a}	302.5^{b}	16.80^{e}	18.3^{c}	11.88^{b}

**Values marked with same letter are not significantly different at p<0.05*

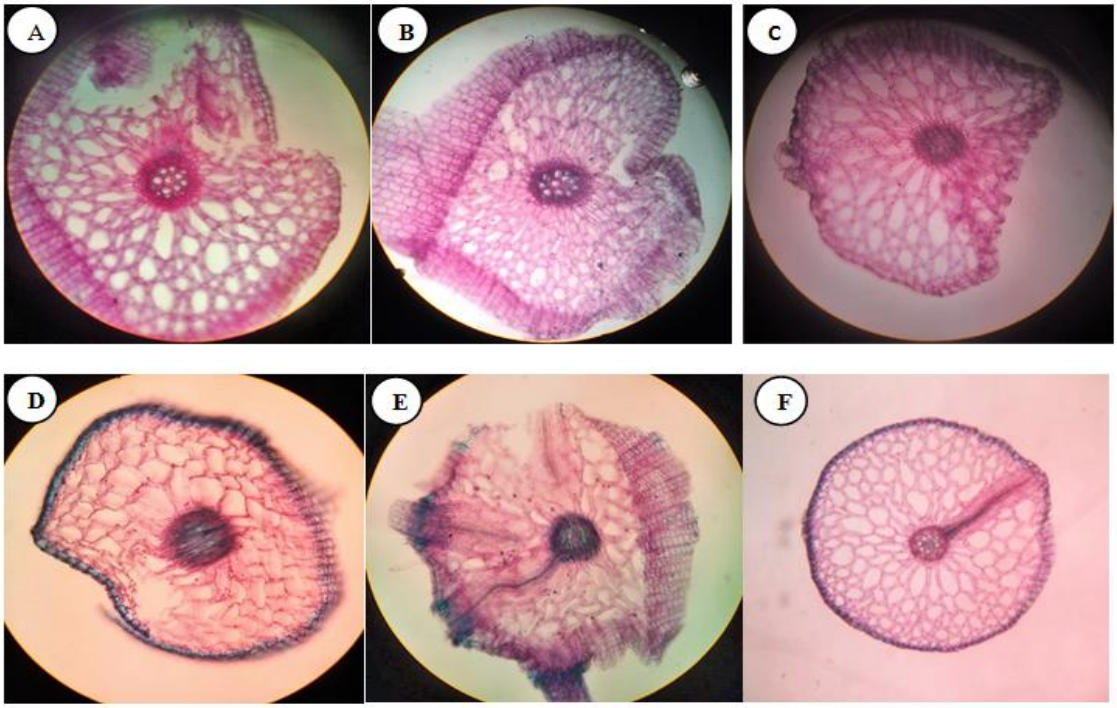

Fig. 2. Root anatomical structure of the six lotus varieties cultivated in Vietnam

A: GiaLong pink lotus; B: PhuMong pink lotus; C: High-yield lotus; D: VinhThanh red lotus; E: Convex white lotus; F: Concave white lotus

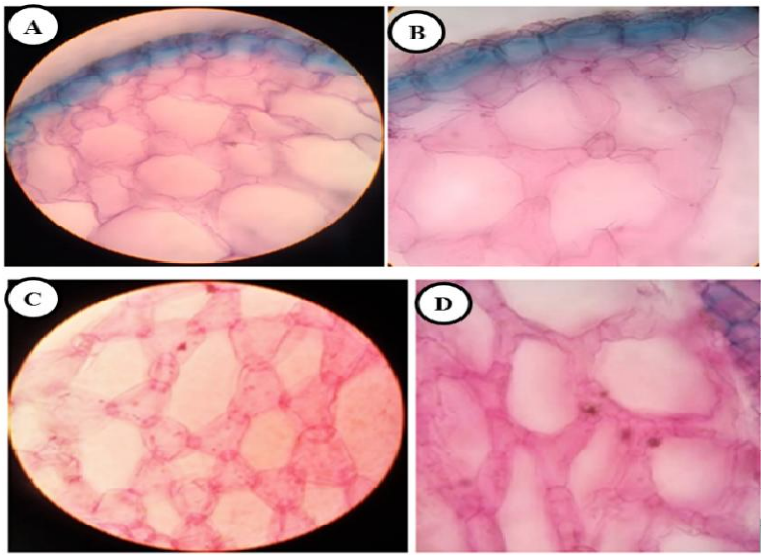

Fig. 3. Epidermis and parenchyma cell of the cortex root of lotus varieties

A: VinhThanh red lotus; B: Concave white lotus; C: High-yield lotus; D: Convex white lotus

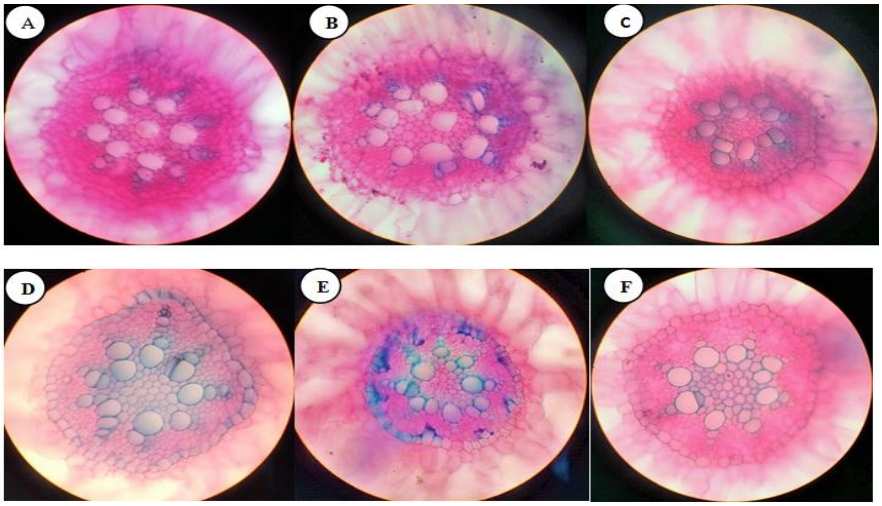

Fig. 4. Anatomical structure of vascular system of the six lotus varieties cultivated in Vietnam

A: GiaLong pink lotus; B: PhuMong pink lotus; C: High-yield lotus; D: VinhThanh red lotus; E: Convex white lotus; F: Concave white lotus

The number of woody vessels is small, about 18.3 – 20.7 vessels/mm^2. The VinhThanh red lotus has the largest number of wood vessels (20.7 vessels/mm^2). The size of woody vessels being about 10.34 - 12.44 µm, while the largest size is found in the PhuMong pink lotus (13.61 µm) and the smallest size being the High-yield lotus (10.34 µm) (Table 1). These findings are consistent with the previous results. According to Thomas et al. [25], the angiosperms have woody vessels whose diameter is 8 -500 µm, depending on characteristics of the different spiecies and the living environment conditions [25]. Thus, the woody vessels diameters of the six lotus varieties

studied are not large, thus promoting the process of water absorption and transferring from the roots to the body quickly.

Anatomical Structure of Rhizomes

The results from studying the anatomical structures of rhizomes show that outside the rhizome is a layer of rectangular epidermis cells which are small in size, regularly arranged, with some of the larger ones in size having curved walls (Fig. 5).

The six lotus varieties are quite homogenous in terms of epidermis size, ranging in 119.70 - 121.80 µm, accounting for 2.77 - 3.06% the rhizome radius (Table 2). The angle collenchymas contains 8 - 10 polygonal cell layers of a non-homogenous size and thin walls; the angle collenchymas consists of starch granules functioning as nutrient storage. The parenchyma consists of polygonal or nearly circular-polygonal cells with thin walls and a non-homogenous size. This contains many starch granules and latex tubes. There are many small and large sponges in the parenchyma. Since the main functionality of the rhizomes is to store nutrients, the parenchyma cells are well developed.

The pericycle of the six lotus varieties consists of rounded parenchyma cells arranged close to each other and some scattered wood vessels. There are large gaps to store and transport air, connected to air canal system inside the lotus, allowing air exchange between the rhizome to the outside environment (Fig. 5). The number of woody vessels is small; the size of woody vessels is large. The six lotus varieties have considerable differences in terms of the pericycle size, in which Gia Long pink lotus has the largest size with 4280.0 µm, account for 97.2% and the smallest size is found in Vinh Thanh red lotus, with 3762.5µm, account for 96.9% (Table 2).

Anatomical Structure of Leaves

Leaf blade: The results in Table 3 show that there is a thick epicuticle covering the upper epidermis of the leaf blades of the six lotus varieties, with the size of 119 - 122 µm, accounting for 8.83 - 11.1% the leaf thickness. There are no significant differences among the six lotus varieties. The size of the lower epidermis is between 66 and 68 µm, accounting for 4.62 - 6.72% the leaf thickness. The Concave white lotus has the largest size (6.72%) and the High-yield lotus has the smallest size (4.62%). Since the lotus leaves are arranged in parallel with the horizontal plane, the lotus leaves always come in contact with the light directly. The thickness of the epicuticle helps prevent heating during the dry season, when the temperature is high.

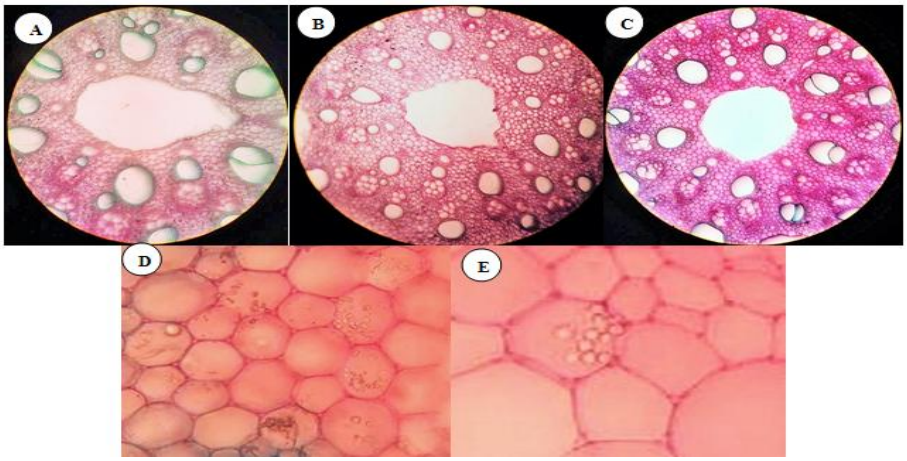

Fig. 5. Rhizomes images from lotus varieties cultivated in Vietnam

A: Rhizomes pericycle of GiaLong pink lotus; B: PhuMong pink lotus; C: High-yield lotus; D: Starch in rhizomes cell of PhuMong pink lotus; E: VinhThanh red lotus.

Table 2. The size of main structural composition of the lotus rhizomes

Variety	Epidermis		Pericycle	
	Mean	Radius (%)	Mean	Radius (%)
GiaLong pink lotus	121.80^{ab}	2.77^{c}	4280.0^{a}	97.23^{a}
PhuMong pink lotus	120.70^{bc}	3.06^{a}	3830.0^{cd}	96.94^{c}
High-yield lotus	120.40^{c}	2.87^{b}	4077.5^{b}	97.13^{b}
VinhThanh red lotus	120.20^{c}	3.10^{a}	3762.5^{d}	96.90^{c}
Convex white lotus	119.70^{c}	2.83^{c}	4122.5^{b}	97.18^{ab}
Concave white lotus	122.80^{a}	3.05^{a}	3907.5^{c}	97.00^{c}

**Values marked with the same letter are not significantly different at p<0.05*

Between the two epidermis layers is an anabolic tissue layer with different thickness levels among the six lotus varieties, varying from 832 - 1238 µm, accounting for 82.12 - 85.87% of the thickness of the leaves. The size of the anabolic tissue is largest in the High-yield lotus with 1238 µm and smallest in the Concave white lotus (832 µm) (Table 3). The thickness of the anabolic tissue helps in enhancing the photosynthesis and the support of leaves.

The palisade parenchyma is only distributed on top of the leaf, which includes the rectangular cell layers. The spongy-parenchyma consists of cells of a non-homogenous size, arranged into many large intercellular air spaces. There are vascular bundles in the leaf vein, which are covered by thick wall cells and sclereid to support the leaf. The woody tissue is less developed (Fig. 6).

Leaf petiole: The results show that components and structural characteristics of the leaf petiole are fairly similar among the six lotus varieties. The epidermis in the petiole includes a one cell layer. The parenchyma and thick epidermis result in thorns of the petiole supporting the leaves. The stellar oxalat canxi crystals are distributed in the parenchymas to increase the mechanical properties of the petiole. Inside the petiole, there is an air space system connected to the leaf vein, leaf blade and stoma for transport. Small vascular bundles are alternately scattered inside the parenchyma layer, below the epidermis layer (Figs. 7, 8, 9).

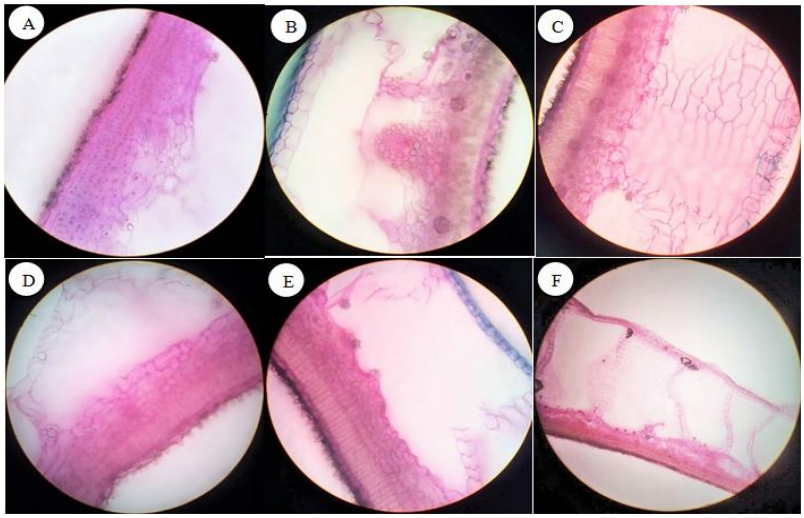

Fig. 6. Microsurgery of blade of the six lotus varieties cultivated in Vietnam

A: GiaLong pink lotus; B: PhuMong pink lotus; C: High-yield lotus; D: Vinh Thanh red lotus; E: Convex white lotus; F: Concave white lotus

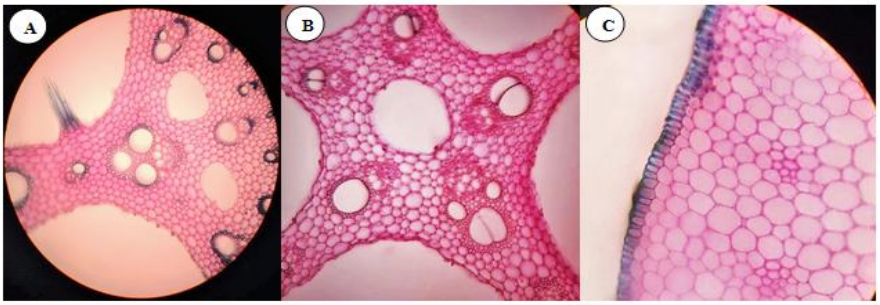

Fig. 7. A: Vascular bundle of GiaLong pink lotus petiole; B: A part of pericycle of High-yield lotus petiole; C: Epidermis and parenchyma cell of Concave white lotus petiole

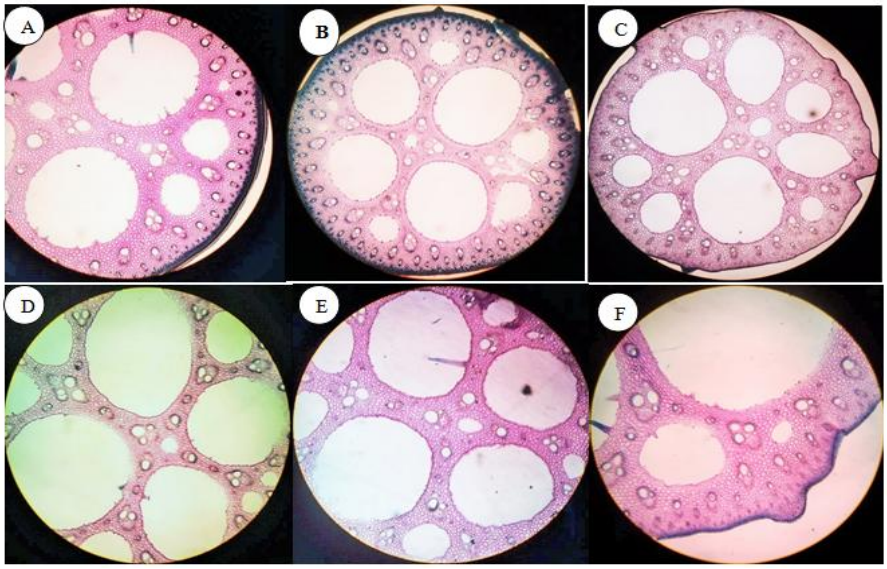

Fig. 8. Microsurgery of petiole of the six lotus varieties cultivated in Vietnam

A: GiaLong pink lotus; B: PhuMong pink lotus; C: High-yield lotus; D: VinhThanh red lotus; E: Convex white lotus; F: Concave white lotus

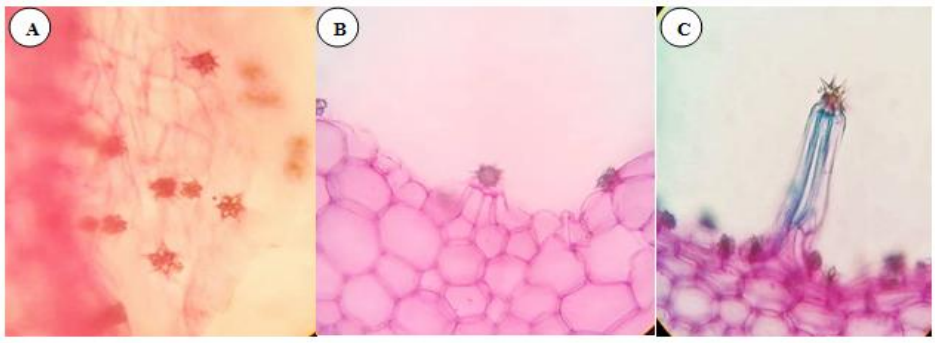

Fig. 9. Calcium oxalate crystals in PhuMong lotus petiole (A) and VinhThanh red lotus (B) and Thorny cells (C)

Table 3. The size of main structural composition of the lotus leaves (µm)

Variety	Upper epidermis		Anabolic tissue		Lower epidermis	
	Mean	Radius (%)	Mean	Radius (%)	Mean	Radius (%)
GiaLong pink lotus	121[a]	8.67[bc]	1207[a]	86.45[a]	68[a]	4.87[bc]
PhuMong pink lotus	119[ab]	9.09[bc]	1125[b]	85.87[ab]	66[a]	5.04[b]
High-yield lotus	126[a]	8.83[bc]	1238[a]	86.55[a]	66[a]	4.62[c]
VinhThanh red lotus	122[a]	8.61[c]	1228[a]	86.52[a]	69[a]	4.86[bc]
Convex white lotus	124[a]	9.24[b]	1151[b]	85.69[b]	68[a]	5.07[b]
Concave white lotus	113[b]	11.10[6a]	832[c]	82.12[c]	68[a]	6.72[a]

**Values marked with same letter are not significantly different at p<0.05*

Air Space Characteristics of Lotus Varieties in ThuaThien Hue Province

Results from the air space characteristics study of the six lotus varieties in ThuaThien Hue are reported in Fig. 10.

Fig. 10 shows that the ratio of the air space area to the petiole cross section area is substantially higher among the six lotus varieties. The ratio is highest in the VinhThanh red lotus, which is 51% and lowest in the PhuMong pink lotus which is 41%. A special characteristic in the lotus structure is the presence of large air spaces connecting to each other, forming an air space system. This air space system connects all parts of the lotus. The study by Volge [26] also shows that there is a special air system in the lotus, starting from the stomata of the leaves, to the stem, to the roots, to the rhizomes; from the roots to the green parts of the lotus; and. finally ending at the leaf center, which is also called the "umbilicus" of the leaf [26]. Because the lotus is one of species that live in an aquatic environment, many parts of the plant can become water logged. This requires the plant to adapt to an anaerobic environment. Hence, the presence of a large air space system helps all parts of the plant so that it can breathe and exchange air with the outside environment in the interconnection among air spaces and the conection with the stomata on the leaf surface. There are many calcioxalat crystals and spongy cells attached by large calcioxalat crystals inside the air space system on the surface of the leaf blade. This helps to insure the solidity of the air space system. This also creates a light and spongy lotus body, allowing lotus plant to be flexible and steady to insure that the leaves are floating out of the water.

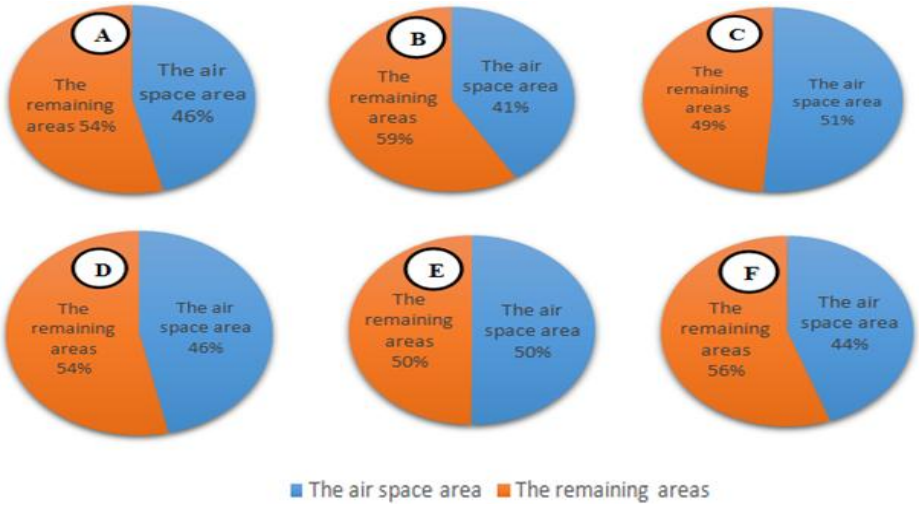

Fig. 10. Air space area/petiole cross section area ratio of the six lotus varieties

A: GiaLong pink lotus; B: PhuMong pink lotus; C: High-yield lotus; D: VinhThanh red lotus; E: Convex white lotus; F: Concave white lotus

CONCLUSION

Results from structural anatomy of the stem, leaf and root of the six lotus varieties show that there is a high level of adaptability of the lotus plant to its aquatic environment. This high level of adaptability has been proven by the spongy roots, the interconnected air space system inside the plant, the high ratio of air space area to blade cross section area (about 41- 51%), and the thick epidermis layer on leaves (about 8.83 - 11.1% the leaf thickness) which helps prevent heating during dry season.

Based on these results it is possible to suggest that the composition of the stem, roots and leaves are similar among the six lotus varieties. The main differences are found in the size and the proportion of this component. This may be due to genetic characteristics of each variety.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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