RESEARCH ON PHYSIOLOGICAL AND ANATOMICAL CHARACTERISTICS OF *AFZELIA XYLOCARPA* (KURZ) CRAIB AT THE NURSERY

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SUMMARY

Afzelia xylocarpa (Kurz) Craib is a high economic value plantation with many significant characteristics such as durable and beautifully patterned wood well tolerated in environmentally stressed conditions. This paper provides preliminary results about anatomical and physiological characteristics of *Afzelia xylocarpa* planting at the nursery. The result shows that *Afzelia xylocarpa* has some interesting phenotypes such as ratio of palisade and spongy parenchyma, 0.71, no multi-epidermis or covered hair. In addition, the result presents the stomata which lies horizontally with the epidermal layer where the average number is 602/mm^2 and the total content of chlorophyll in fresh leaves is 10.42 mg/g with the ratio of a/b chlorophylls 1.49. The physiological data illustrates the evapotranspiration intensity, 0.63 g/dm^2/h, and the water absorption figure, 14.09 atm. Moreover, the result also indicates that different temperatures (45, 50 and 60^{0}C) had a different effect on leaf tissues that was demonstrated by damaged/fresh ratios, 14.5%, 72% and 100%, respectively. All the above evidence suggests that *Afzelia xylocarpa* may need a medium intensity of light, would lose water easily and shows weak ability to water adsorption. These might play important roles in planting *Afzelia xylocarpa* in the future.

Keywords: *Afzelia xylocarpa,* anatomical characteristics, chlorophyll, physiological characteristics, stomata.

1. INTRODUCTION

Afzelia xylocarpa (Kurz) Craib, known as te or Ho bi or Teakha, belongs to *Fabaceae* family, *Caesalpinoideae* sub-family. They are big plants at 30 meters high with a trunk diameter, reaching 2 meters. They distribute at evergreen or semi-deciduous forestry, wet or semi tropical condition zones. They have been found in Lao (Bolykhamxay, Vienchan city), Vietnam (Kon Tum, Gia Lai, Dac Lac, Khanh Hoa and East-Southern provinces), Thailan and Myanmar. In nature, they were distributed not in a population but in an individual plant among other species in the forestry (Nguyen Hoang Nghia, 1999).

According to Vietnam's Red Book (2007), *Afzelia xylocarpa* was classified as EN A1c, d. Their wood is very popular in the market, especially in Lao, because of the flexibility in many applications such as house building, furniture, high-quality hand-made furniture (Nguyen Duc Thanh *et al*., 2012, Nguyen Duc Thanh, 2016). Notably, the numbers of *Afzelia xylocarpa* in natural forestry are very low and is endangered for sources of important genes from native plants (Nguyen Hoang Nghia, 1999).

Currently, there are some studies for this plant reported. However, most of them studied on phenotypical and eco-systemic characteristics and not yet on anatomical and physiological characteristics. These lead to problems to optimize the environmental conditions for planting at the nursery stage of *Afzelia xylocarpa*. Therefore, to get more scientific knowledge that may help in breeding *Afzelia xylocarpa*, we carried out research on the anatomical and physiological characteristics of this plant.

2. RESEARCH METHODOLOGY

2.1. Material

Fresh leaves of 6-month old *Afzelia xylocarpa* plantlets growing in a nursery , being planted in the College of Forestry Biotechnology, Vietnam National University of Forestry.

2.2. Methods

Anatomy: leaf samples of three different species were randomly chosen., Three different positions on each leaf sample were anatomized and data collected of the total number of stomata on under-leaf surfaces or the thickness of layers under the OPTIKA M-699 microscope link Optikam PRO 3 Digital Camera.

Identify the content and ratio of chlorophyll a and b as described in Benz *et al.* (1980).

Identify the evaporation intensity of water as method described in Ivanov *et al.* (1950).

Identify water adsorption of plant tissues as methods described by Shacdacov: Briefly, two series of 10 falcon tubes was prepared, one for standard curve and one for experiment, which is containing NaCl solution with concentration from 0.1 to 1M (0.1M difference). Consequently, two parallel tubes had the same concentration. Then, 2 and 3mL of NaCl solution were added into the series of standard and experimental tubes, respectively. In the next step, we used a wine-opener to collect pieces of samples and placed 5 pieces per tube. Then, these tubes were incubated at room temperature for 30 minutes and occasionally mixed by gentle shaking. After that, the pieces of samples were taken out and we added 1 drop of methylene blue. The mixture was well shaken and a pipet used to suck up the solution from experimental tubes and slowly poured into parallel standard tubes. During this step, the movement of blue solution was carefully observed to find a concentration in which the blue solution did not move meaning the concentration of the cytoplasm of cell (C_{tb}) is equal to the one of NaCl solution (C_{dd}). This concentration is called the isotonic concentration. Hence, the water adsorption of cell (S_{tb}) is equal to the one of isotonic solution (S_{dd}).

$$S_{tb} = S_{dd} = R.\ T.\ C_i$$

In which: S_{tb}: water adsorption of cell; S_{dd}: water adsorption of isotonic solution; R = 0,0821 = air constants; C: the concentration of the cytoplasm of cell; i: isotonic constants; i = 1 + α (n-1); α: electronic fraction; n: ion numbers after an electronic reaction.

Identify the heat tolerance by using methods of Maxcop: Briefly, water was boiled and a series of cups were prepared to contain water with different temperatures: 35^0 C, 40^0 C, 45^0 C, 50^0 C, 55^0 C, 60^0 C. Then, leaf samples were immersed in these cups for 30 minutes. After that, the immersed samples were transferred into a cup containing room-temperature water for 2 - 5 minutes. Then, the water was discarded and added HCl 0.2 N solution. The mixture was incubated for 20 minutes before taking out the leaf samples to measure the damaged area (%).

Experiments: 1) ĐC: No sunlight; 2) CS_1: 25% shaded; 3) CS_2: 50% shaded; 4) CS_3: 75% shaded.

3. RESULTS AND DISCUSSIONS

3.1. Leaf anatomy

Epidermis and Curtin are outside layers of leaf that play important roles in the protection and reduction of water evaporation for inner layers. In addition, they also carry out many other processes. In addition, the size of epidermis cells or the thickness of the curtin layer is evidence for the adaptation with non-desired environmental conditions such as highly intensive sunlight. Hence, studying the epidermis layer plays an important role in planting and caring for *Afzelia xylocarpa* plantlets. Interestingly, the result shows that both sides of a leaf of *Afzelia xylocarpa* do not have multi-layer epidermis and the Curtin-layer thickness is relatively constant suggesting the light adsorption is the same on both sides of a leaf (Table 1 and Figure 1).

Table 1. Anatomic analysis of a leaf

Exp.	Average value of anatomic factors (*µm*)							MD/ MK	Total stoma/mm^2
	CTT	BBT	MD	MK	BBD	CTD	Leaf thickness		
ĐC	3.28	16.25	36.06	49.10	12.56	3.50	120.75	0.73	616
CS_1	3.25	15.74	32.76	45.85	12.04	4.38	114.02	0.71	604
CS_2	3.16	10.80	32.08	45.64	9.04	3.03	103.75	0.70	599
CS_3	3.09	10.55	29.48	43.34	8.92	2.91	98.29	0.68	589
Average	***3.20***	***13.33***	***32.59***	***45.98***	***10.64***	***3.45***	***109.20***	***0.71***	***602***

Notes: CTT: upper cutin; BBT: upper epidermis; MD: palisade layer; MK: spongy layer; BBD: lower epidermis; CTD: lower cutin; KK: stomata.

The results described in Table 1 presents the MD/MK ratio of about 0.71, suggesting the above medium requirement of light for *Afzelia xylocarpa* leaves. In addition, the anatomic analysis also shows that both layers of epidermis do not have covered hairs, which are dead cells containing air and play significant roles in light reflection and heat relief of leaves. Furthermore, the stomata located horizontally with epidermis cells and average number of stomata per 01 mm^2 is 602, which is different from other plants such as *Manglietia conifera* (199/mm^2), *Erythrophleum fordii* (464/mm^2), red eucalyptus (486/mm^2), *K. senegalensis* (929/mm^2), and white eucalyptus (420/mm^2) (Nguyen Thi Tho *et al.*, 2013).

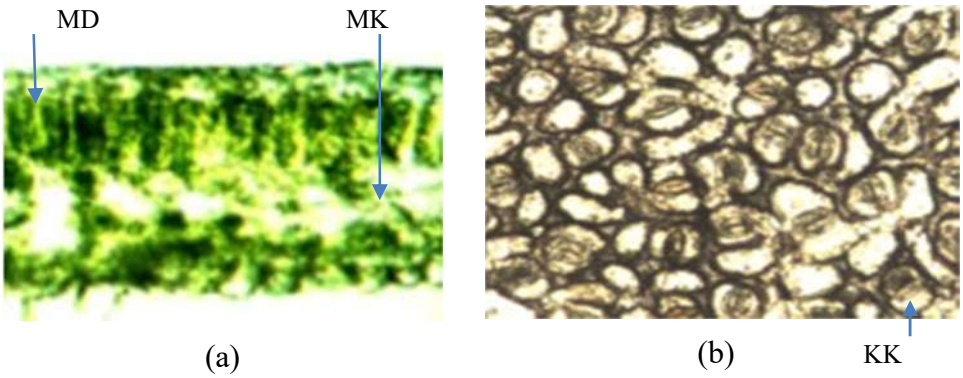

Figure 1. Leaf anatomic picture

(a) Cross section of leaf blade ; b) Shape and number of stomata)

3.2. Qualitative analysis of chlorophyll from leaves of *Afzelia xylocarpa*

Chlorophylls play important roles in plant synthesis, which produce inorganic compounds from carbon dioxide and water with the catalysis of sunshine. Interestingly, many studies have been done on photophilic plants and demonstrated that photophilic plants have a small number of chlorophylls and a high ratio of chlorophyll a/b (about 5.5). On the other side, the photophobic plants shows opposite features such as a high number of chlorophyll and low chlorophyll a/b ratio (≤ 1.4). Therefore, the light demand of a plant can be demonstrated by two factors including the chlorophyll amount and ratio of chlorophyll a/b (Vu Van Vu *et al.*, 2000).

To find out the light demand of *Afzelia xylocarpa* plantlets, we analyzed the chlorophyll amount in leaf samples collected from differently shaded plantlets. The results are shown in table 2.

Table 2. Amount of chlorophyll in a leaf of *Afzelia xylocarpa* plantlet

Exp.	Amount of chlorophylls (mg/g)			Ratio of chlorophyll (a/b)
	Chlorophyll a	Chlorophyll b	Total	
ĐC	5.92	4.01	9.94	1.76
CS_1	6.25	3.99	10.23	1.57
CS_2	6.20	4.19	10.39	1.48
CS_3	5.90	5.21	11.11	1.13
Average	***6.07***	***4.35***	***10.42***	***1.49***

As can be seen from table 2, the average amount of chlorophyll a and b from *Afzelia xylocarpa* leaf is 6.06 and 4.35 mg/g, respectively. The total amount of chlorophyll is 10.42 mg per gram of fresh leaf and the chlorophyll (a/b) ratio is 1.49. These data illustrate that *Afzelia xylocarpa* plantlet adapts in medium sunlight.

3.3. Evaporation intensity of leaves

The experiments were carried out at The College of Forestry University, Vietnam National University of Forestry. Environmental conditions in this location include light intensity (3000 lux), humidity (77%), temperature (30^0C). The data were collected, analyzed, and presented as in table 3.

Table 3. Water evaporation intensity and water adsorption force of leaves

Exp.	Water evaporation intensity (g/dm^2/h)	water adsorption force (atm)
ĐC	0.69	17.21
CS_1	0.66	15.15
CS_2	0.64	13.06
CS_3	0.51	10.95
Average	***0.63***	***14.09***

3.3.1. Evaporation intensity of water

It is already known that the water evaporation of leaves plays important roles in the cooling down process of plants and in generating forces to transfer water and minerals from roots to the leaves. Hence, analyzing the water evaporation of leaves can reveal the demand of water and minerals for a plant as well as the plant development. In this paper, the results showed that an average value of water evaporation intensity is 0.63 g/dm^2/h presenting the slow evaporation of leaf samples (Table 3). The results are consistent with previous studies that suggest medium requirement of sunlight for plantlets of *Afzelia xylocarpa*.

3.3.2. Water adsorption force of leaf cells

Drought tolerance of plants seriously involves to concentration of metabolites in the cell cytoplasm, which plays significant role in producing adsorption force for roots. The results are described as in table 3. As can be seen, the water evaporation force of leaf cells is about 14.09 atm. This value is smaller than the one of drought-tolerance plants such as *Casuarina Adans* (19.86 atm) suggesting plantlets of *Afzelia xylocarpa* have just above medium demand for water. This is consistent with the results that presented the low evaporation of leaves (*3.3.1 section*).

These figures together with the anatomic results demonstrated that the plantlets of *Afzelia xylocarpa* easily lose water through evaporation of leaves but their ability in water adsorption is very low. This leads to problems

in planting and caring for plantlets in dry and hot conditions.

3.4. Heat tolerance of *Afzelia xylocarpa*

Temperature, sunlight and humidity play significant roles on annual, seasonal, or day and night activities of organisms. High temperature showed functions in producing regional distribution and classification of plants. Hence, studying effects of temperature on plantlets is very necessary. The results were presented in table 4.

Table 4. Heat tolerance of *Afzelia xylocarpa*

Exp.	Damaged percentage of leaves (%)					
	35^0 C	40^0 C	45^0 C	50^0 C	55^0 C	60^0 C
ĐC	0	1	12	66	99	100
CS_1	0	3	14	69	100	100
CS_2	0	6	15	74	100	100
CS_3	0	7	17	79	100	100
Average	***0***	***4.25***	***14.50***	***72.0***	***99.75***	***100***

35^0 C 40^0 C 45^0 C 50^0 C 55^0 C 60^0 C

Figure 2. Percentage of damaged area of leaves under different temperatures

As can be seen, leaf samples have low heat tolerance (Figure 2): no damaged leaves were found at 35^0C, but when the temperature rose up to 40^0C, many small damaged areas appeared. Notably, at 45 and 50^0C, the percentage of damaged areas dramatically increased reaching 14.5 and 72%. Moreover, the leaf color changed from green into light brown at 50^0C. This result suggests the cell membranes were damaged leading to HCl entering and causing a damage of cytoplasm components and chlorophylls. In addition, the results also showed that 99.75% of a leaf have been turned into dark brown with a very small green area at 55^0C, and especially, there is no longer any green color at 60^0C. As previous studies at Nui Luot location, the temperature can reach to 43^0 C on June and average temperature is 23.1^0C. All of these suggest the leaves can be affected in June and may lead to defects in plant development.

4. CONCLUSION

Through the physiological and anatomical experiments of leaves of *Afzelia xylocarpa*, our results showed that: 1) The leaves do not have multiple layer epidermis and covered hair. In addition, the ratio of palisade layer/ spongy layer is about 0.71; 2) The stomata lies horizontally with epidermis cells and an average number of stomata is 602/mm^2; 3) The amount of chlorophyll in a and b from fresh leaves is 6.07 and 4.35 mg/g, respectively; 4) Water evaporation intensity is 0.63 g/dm^2/h and water adsorption force are 14.09 atm; 5) The percentage of damaged area is 14.5, 72 and

100% at 45, 50 and 60^0 C, respectively. This data suggests that *Afzelia xylocarpa* has a medium requirement of light and might be useful for further experiments in future.

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NGHIÊN CỨU MỘT SỐ ĐẶC ĐIỂM SINH LÝ VÀ GIẢI PHẪU LOÀI GÕ ĐỎ (*AFZELIA XYLOCARPA* (KURZ) CRAIB) TUỔI VƯỜN ƯƠM

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TÓM TẮT

Gõ đỏ (*Afzelia xylocarpa* (Kurz) Craib) là cây có giá trị kinh tế cao do có gỗ tốt, bền và hoa văn đẹp, chịu đựng tốt với môi trường. Bài báo này công bố kết quả nghiên cứu một số đặc điểm sinh lý và giải phẫu lá Gõ đỏ. Kết quả nghiên cứu cho thấy, không thấy xuất hiện biểu bì nhiều lớp và lông che chở ở hai mặt của lá. Tỷ lệ mô dậu/mô khuyết trung bình là 0,71, điều đó nói rằng mẫu Gõ đỏ nghiên cứu có nhu cầu ánh sáng ở mức trung bình khá. Khí khổng nằm ngang so với bề mặt của biểu bì, số lượng khí khổng bình quân là 602/mm^2. Hàm lượng diệp lục a và b thu được trong lá tươi lần lượt là 6,07 và 4,35 mg/g; hàm lượng diệp lục tổng số trung bình là 10,42 mg/g lá, tỷ lệ diệp lục a/b lại không cao chỉ khoảng 1,49. Như vậy, mẫu Gõ đỏ đang nghiên cứu thích ứng với điều kiện ánh sáng trung bình thấp. Cường độ thoát hơi nước của Gõ đỏ bằng 0,63 g/dm^2/h, sức hút nước của tế bào bằng 14,09 atm. Gõ đỏ bị tổn thương 14,5% ở mức nhiệt 45^0C và mức tổn thương đến 72% ở mức nhiệt 50^0C, chúng bị chết hoàn toàn ở mức nhiệt 60^0C.

Từ khóa: Chịu nóng, diệp lục, đặc điểm giải phẫu, đặc điểm sinh lý, Gõ đỏ, thoát hơi nước.

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