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THE FEATURES OF REPRODUCTIVE ADAPTION OF CARNIVOROUS PLANT *Utricularia aurea*

Duong Thi Minh Hoang
Faculty of Primary Education, University of Education, Hue University

Abstract. Carnivorous plant *(Utricularia aurea*) is a suspended aquatic. Inflorescence erect, glabrous, scales absent, bracts basifixed, broadly ovate to orbicular. Calyx lobes ovate, subequal, apex rounded to subacute; lower lobe often slightly broader than upper lobe. Corolla pale yellow, pubescent or glabrous; lower lip transversely elliptic, base with a prominent 2-lobed swelling, apex rounded to emarginate. Filaments 1- 1.5 mm, curved; another thecae confluent. Ovary ovoid; style evident; stigma lower lip semicircular, upper lip obsolete. Fruit**:** capsule, ovoid, many seeds. The pollen of *Utricularia aurea* has one morphological form: heterocolpate (polar view). The diameter of fertile pollen is 32,97 ± 0,20 µm, the diameter of infertile pollen is 28,17 ± 0,20 µm. The percentage of fertile pollen is low (< 50%): 26,92% (in peak flowering) and 23,94% (in rare flowering). The percentage of fertile pollen has a positive and close correlation with the diameter of pollen (R = 0,95). Although this species has two reproductive forms (vegetative and sexual), the efficiency of sexual reproduction isn't high. Vegetative reproduction is always dominant.

Keywords: *Utricularia aurea*, pollen, percentage of fertile pollen, fertile pollen, infertile pollen, vegetative reproduction, sexual reproduction.

1. Introduction

People often know Carnivorous plants by special catabolism and interesting shapes. The genus Utricularia, family Lentibulariaceae, is the largest genus of carnivorous plants. Aquatic species in this genus are noticed by humans for their traps. People call them Bladderworts. Therefore, most studies have focused on exploring the diversity of morphology and mechanism of action of traps [1, 2].

However, not many people realize that adaptive changes in morphological and physiological characteristics are not enough to survive in a harsh environment. These species must maintain the race and pass on to the next generation these characteristics. Therefore, the formation of specific reproductive methods is also an indispensable thing. In other words, vegetative adaption and reproductive adaption always go hand in hand and determine the survival of the species.

Utricularia aurea is bladderwort, and also an aquatic species at waterbodies of Vietnam. Therefore, the study of the reproductive biology of this species will complement studies of the genus *Utricularia* and help us understand more about the reproductive adaptations of plants to submerged environments. know their reproductive mechanism. On that basis, we can actively control the balance of the wetland ecosystem.

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Contact Duong Thi Minh Hoang, e-mail address: dtmhoang@hueuni.edu.vn

2. Content

2.1. Research objects and methodology

**** Research objects***

Fresh flowers and pollens of *Utricularia aurea* (Figure 1) were collected in wetlands and water fields at Tram Tra Loc, Hai Lang district, Quang Tri province. They were collected at different times of the year. The number of flowers at each collection was just large enough to be able to analyze flower morphology and structure as well as analyze pollen grains (n > = 30).

**** Research Methodology***

** Determination of pollen morphology and pollen size*

Pollen grains were selected from newly hatched flowers of *Ultricularia aurea* and then dyed with aceto-carmine 5%. The anthers were separated, crushed on a small plate, and diluted with water. The pollen morphology was observed in the 40x objective of Olympus CH20 optical microscope.

The method of measuring pollen size was conducted according to John K. Kelly et al. (2002) [3]. 30 pollen grains were measured before they were dyed and 30 pollen grains were measured after they were dyed. The experiment was repeated 5 times with an objective microscope and OMII eyepiece (in 40x objective).

** Determination of the percentage of fertile pollen*

Pollens were dyed with aceto-carmine 5% in order to identify fertile pollen and infertile pollen based on Reijieli R. Rigamoto et al. (2002) [4]. Fertile pollen turned into deep red, while infertile pollen was light or not colored. 10 fields were selected randomly from each template to observe under 10x objective (100 times magnification). The percentage of fertile pollen was the percentage of the total of fertile pollens in the total of pollens counted in the field.

The percentage of fertile pollen was calculated based on the following formulation:

$$\text{The percentage of fertile} = \frac{\text{the total of fertile pollens}}{\text{the total of pollens counted}} \times 100\%$$

** Determination of the number of pollens*

The number of pollens was determined by using the red blood cell Goriaep. The solution containing pollens was added to the counting chamber. The number of fertile pollens and the number of infertile pollens were then counted.

** Observation of vegetative reproduction and seed reproduction*

The number of shoots was generated from vegetative and seed reproduction (sexual reproduction) in the standard plots, with n = 10 cells, each cell was $1m^2$ respectively.

** Methodology of evaluation of seed germination*

100 seeds were sown in white sand which was taken from the study site. Then, the number of germinating seeds and the number of non-germinating seeds were counted.

** Methodology of morphological analysis of flower structure*

Plants in the field were taken photos. Also, flower samples were collected and analyzed by the dissecting microscope.

** Data analysis*

Research data were statistically analyzed by MS Excel 2016 software.

2.2. Research results

2.2.1. The structure of flower and fruit

Utricularia aurea is a suspended aquatic carnivorous plant, it is cardamom, submerged in water that has a long and slender body with little branching. Its leaf is fibrous in shape, 4 - 5 cm long, carrying many digestive bags (traps) leaf segments.

Inflorescence: erect, 5 - 25 cm, 3 - 7 flowered, glabrous, scales absent, bracts basifixed, broadly ovate to orbicular (Figure 1). Calyx lobes ovate, subequal, apex rounded to subacute; lower lobe often slightly broader than upper lobe. Corolla pale yellow, 1 - 1.5 cm, pubescent or glabrous; lower lip transversely elliptic, base with a prominent 2-lobed swelling, apex rounded to emarginate. Filaments 1 - 1.5 mm, curved; another thecae confluent. Ovary ovoid; style evident; stigma lower lip semicircular, upper lip obsolete. Fruit**:** capsule, ovoid, many seeds, about 78 - 153 seeds.

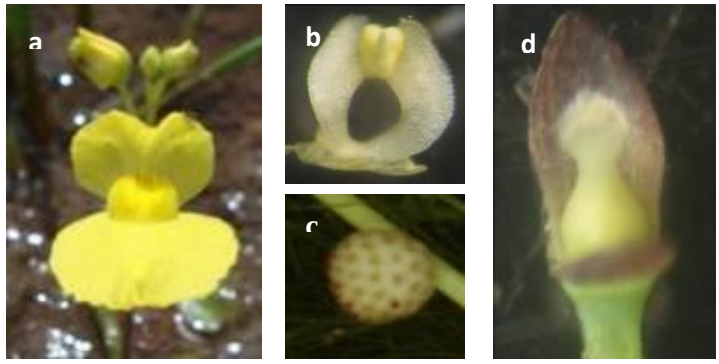

Figure 1. Reproductive organs of Utricularia aurea
a. Inflorescence; b. Anther; c. Fruit; d. Stigma

2.2.2 The morphology and size of pollen

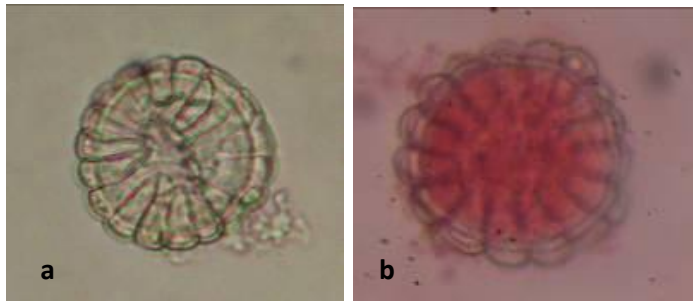

Figure 2. Pollen of (Utricularia aurea) in polar view
a. Infertile pollen b. Fertile pollen

Looking from the pole, pollens have a round shape, divided into many lobes, cut deep into the inside. In another word, the pollen of *Utricularia aurea* has one morphological form: heterocolpate (polar view) (Figure 2). This character is similar to 7 species in the same genus Utricularia in the study of Mario Beretta et al. (2014) [5].

The average diameter of fertile pollen is 32.97 ± 0.20 µm, while the average diameter of infertile pollen is: 28.17 ± 0.20 µm (Table 1).

Table 1. The average diameter of fertile pollen and infertile pollen

Fertile pollen		**Infertile pollen**	
Amplitude (µm)	The average diameter (µm)	Amplitude (µm)	The average diameter (µm)
25,00-32,50	32,97 ± 0,20	28,75-37,50	28,17 ± 0,20

The data in Table 1 showed that the average diameter of fertile pollen was larger than that of infertile pollen. This research result was consistent with the study of Jovanka Atlagic et al (2009) [6]; Tran Quoc Dung et al. (2009) [7]. According to Jovanka Atlagic & cs., while the diameter of infertile pollen was distributed between 15 - 40 µm and the highest from 15 -35 µm; the fertile pollen was in the range of 20 - 60 µm and the highest was from 35 - 60 µm [6]. This result also showed that fertile pollens were larger in size than infertile pollens. The correlation between the size and the percentage of fertile pollen would be further analyzed in section 3.4.

2.2.3. The percentage of fertile pollen

After being dyed, grains were counted and then the percentage of fertile pollen was measured at 2 times: the time when flowers scatter (early season and end of the season) and the time when flowers bloomed. Research results were summarized in Table 2.

The percentage of fertile pollen of *Utricularia aurea* at the time of blooming flowers was higher than the time of scattered flowers. This suggested that the percentage of fertile pollen was not only determined by genetic factors, but also by environmental conditions.

The number of fertile pollens of *Utricularia aurea* at both times was lower than that of infertile pollens, indicating that the sexual reproduction of this submerged species was limited. This was consistent with the reproductive nature of *Utricularia aurea* species in reality [8].

In addition, Ahmad's research results (2010) on the percentage of fertile pollen of 46 terrestrial species showed that except for *Spergularia arvensis*, the percentage of fertile pollen reached 66.67%, while the rest had the percentage of fertile pollen with over 70%, some species even reached 100% [9]. Meanwhile, the study by Duong Thi Minh Hoang (2011) represented that the percentage of fertile pollen of some submerged plants was all below 60% [8].

Thus, it can be said that the ratio of the low percentage of fertile pollen (<50%) is one of the adaptive forms of *Utricularia aurea* in particular and of the plants living in the water environment in general.

Table 2. The percentage of fertile pollen is 2 times: flowers scatter and flowers bloom

Time / **Genre of pollen**	**Flowers scatter**		**Flowers bloom**	
	Quantity	***The percentage (%)***	***Quantity***	***The percentage (%)***
Fertile pollen	163	23,94	189	26,92
Infertile pollen	518	76,06	513	73,08

2.2.4. The correlation between the size and the percentage of fertile pollen

In order to assess the correlation between the size and the percentage of fertile pollen, the author used Excel 2016 software and the distribution of experimental data, then determined the

correlation coefficient (R). At the same time, the author estimated the regression equation of the relationship.

The correlation coefficient R was evaluated based on the arrangement scale of Nguyen Hai Tuat & Ngo Kim Khoi (1996) [10] as follows:

R = 0: x and y are independent of each other.

R = 1: x and y have a functional relationship.

0 < | R | ≤ 0.3: the correlation between x and y is weak.

0.3 < | R | ≤ 0,5: the correlation between x and y is not strong.

0.5 < | R | ≤ 0,7: the correlation between x and y is relatively close.

0.7 < | R | ≤ 0,9: x and y are closely related.

0.9 < | R | <1: x and y have a very close correlation.

The research results on diameter and the percentage of fertile pollen of Utricularia aurea were shown in Table 3.

Table 3. Diameter and the percentage of fertile pollen of Utricularia aurea

Diameter (µm)	The percentage of fertile pollen (%)	Diameter (µm)	The percentage of fertile pollen (%)
25	0	32,5	90
26,25	0	33,75	100
27,5	0	35	100
28,75	18,18	36,25	100
30	39,29	37,5	100
31,25	54,55	-	-

The data in Table 3 demonstrated that the percentage of fertile pollen was maximum (100%) when pollens were large in size from 33.75 to 37.5 µm. In contrast, the author did not find any pollen grain in small size from 25 - 27.5 µm. Similar to the above cases, between 28.75 - 37.5 µm, the percentage of pollen gradually increased with the large size of pollens.

When examining the correlation coefficient between the percentage of fertile pollen and the pollen's diameter of *Utricularia aurea* species, the results obtained are R = 0.95. This represented a very close relationship with linear regression equation y = 10,28x - 266.7 (Figure 3).

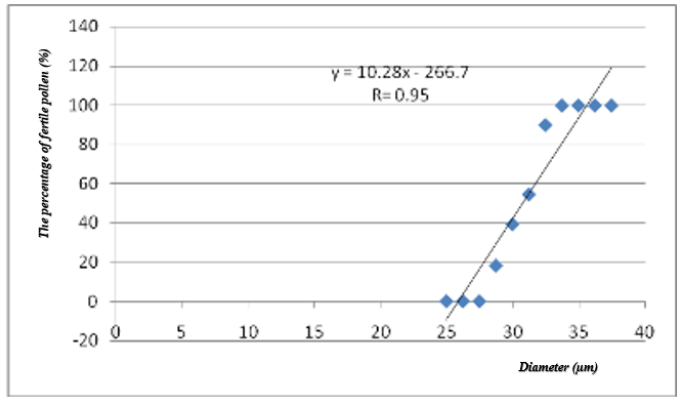

Figure 3. the correlation coefficient between the percentage of fertile pollen and the pollen's diameter of Utricularia aurea

In conclusion, in *Utricularia aurea*, the percentage of fertile pollen had a strong and close correlation with the pollen's diameter. This result was consistent with the research of Hiroaki Inoue & et al. (1992) in *Persea americana* [11]; John K. Kelly et al. (2002) in 2 species: *Mimulus guttatus* and *Collinsia verna* [6] and Hoang Xuan Thao & Le Thi Tre (2009) in *Lumnitzera rosea* [12]. These authors all agreed that the fertile pollens were larger in size than the infertile pollens.

2.2.5. Reproductive ability

In nature, *Utricularia aurea* reproduced vegetatively with the body. *Utricularia aurea* had an average of 1.4 shoots/ individual. In theory, the sexual reproduction ability of *Utricularia aurea* was very high: 103.17 ± 2.82 grains. That meant one seed would form one seedling, but the actual number of seedlings regenerated from the seed was very small, only 0.8 ± 0.25 plants in a standard plot. This exhibited that the reproductive efficiency of *Ultricularia aurea* 's seeds was very poor. Perhaps due to living in submerged environments, the dispersal and germination of seeds were not favorable for the following reasons: seeds buried under mud or destroyed by microorganisms [13]. Hence, *Utricularia aurea* had both forms of reproduction: vegetative reproduction and seed reproduction. However, seed reproduction was much lower than vegetative reproduction.

3. Conclusions

The following are the conclusions drawn from the study of the adaptive features of *Utricularia aurea* reproduction: Looking from the pole, pollens have a round shape, divided into many lobes, cut deep into the inside. That means the pollen of *Utricularia aurea* has one morphological form: heterocolpate (polar view). The average diameter of fertile pollen is: 32.97 ± 0.20 µm, the diameter of infertile pollen is: 28.17 ± 0.20 µm. The percentage of fertile pollen is low (<50%): 26.92% (in the flowering season) and 23.94% (in the season of scattered flowers). The percentage of fertile pollen has a positive and close correlation with pollen's diameter (R = 0.95). In this species, there exists both vegetative reproduction and seed reproduction. However, vegetative reproduction is still much more dominant than seed reproduction.

REFERENCES

[1] Westermeier, A.S., Fleischmann, A., Müller, K. et al, 2017. Trap diversity and character evolution in carnivorous bladderworts (*Utricularia*, Lentibulariaceae). *Scientific Reports 7*, 12052.

[2] Lubomír Adamec, 2011. Function characteristics of traps of aquatic carnivorous Ultricularia species. Aquatic Botany, Vol. 95, Issue 3, pp. 226-233.

[3] Kelly J. K., Rasch A. & Kalisz S., 2002. A method to estimate pollen viability from pollen size variation. *American Journal of Botany*, Vol. 89(6), pp. 1021-1023.

[4] Rigamoto R. R., Tyagi A. P., 2002. Pollen Fertility Status in Coastal Plant Species of Rotuma Island. *The South Pacific Journal of Natural Science*. The University of the South Pacific, Vol. 20, pp. 30-33.

[5] Mario Beretta, Graziella Rodondi, Lubomír Adamec, Carlo Andreis, 2014. Pollen morphology of European bladderworts (*Utricularia* L., Lentibulariaceae). *Palaeobotany and Palynology*, Vol. 205, pp. 22-30.

[6] Jovanka A., Ana M.J., Radovan M. & Sreten T., 2009. Pollen grain traits of oil species from the Novi Sad collection. *Genetika*, Vol. 41(3), pp. 263-270.

[7] Tran Quoc Dung & Nguyen Khoa Lan, 2009. Determine the size and the percentage of fertile pollen of *Lemnitzera racemosa* Wild.1803). *The third national scientific conference on ecology and biological resources.*

[8] Duong Thi Minh Hoang, 2011. *Research on reproductive phenology of some submerged plants in Tram Tra Loc, Hai Lang district, Quang Tri province.* Master's Thesis, University of Education, Hue University.

[9] Ahmad K., Shaheen N., Ahmad M. & Khan M. A., 2010. Pollen fertility estimation of some sub-tropical flora of Pakistan. *African Journal of Biotechnology*, Vol. 9(49), pp. 8313-8317.

[10] Nguyen Hai Tuat & Ngo Kim Khoi, 1996. *Statistical processing of empirical research results in agro-forestry on computers (by excel 5.0)*, Hanoi Agriculture Publisher, p. 79.

[11] Inoue H., Takahashi B. & Shirato K., 1992. Morphology and Fertility of Pollen of Avocado (*Persea americana* Mill.). College of Agriculture and Veterinary Medicine, Nikon University, Fujisawa, Kanagawa, 252 Japan.

[12] Le Thi Tre & Hoang Xuan Thao, 2009. The percentage of fertile pollen and the dipoid chromosome set of *Lemnitzera rosea* (Gaud) Presl. 1834. *The third national scientific conference on ecology and biological resources.*

[13] Barrett S.C.H., 1980. Sexual reproduction in *Eichhornia crassipes* (Water Hyacinth). *Journal of applied ecology, Blackwell scientific publications*, Vol. 17, pp. 113-124.