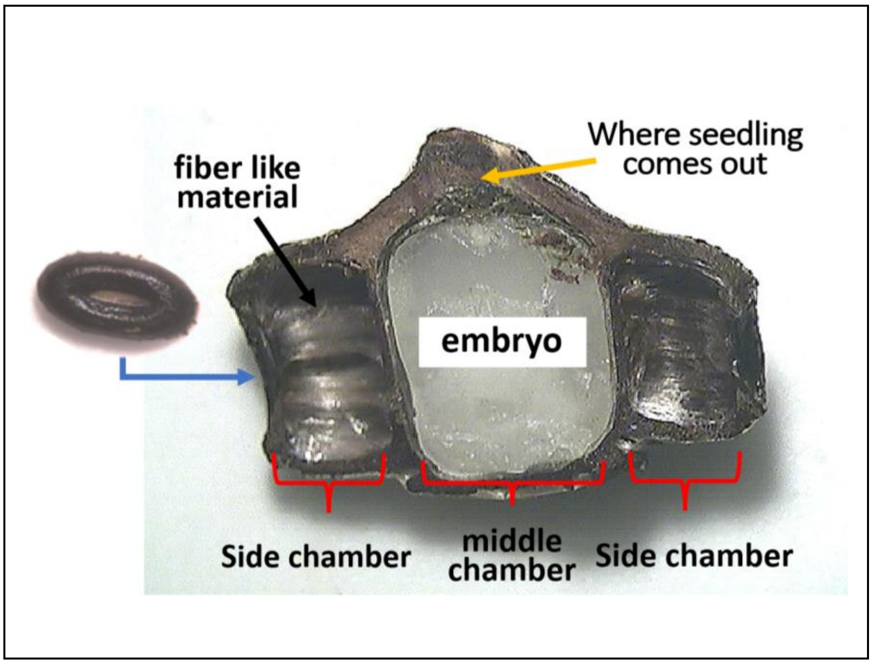
DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES

UNIQUE SHAPE OF TRICHOSANTHES PILOSA SEED AND ITS BIOLOGICAL MEANING



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Cover photo: Akari Bergquist, 2018, structure inside *Trichosanthes pilosa* seed

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Abstract

Trichosanthes.pilosa(=T. pilosa) commonly known as Japanese snake gourd is a plant in Cucurbitaceae family. They are adapted to grow in tropical monsoon, wet tropical, and humid subtropical climates across regions in East Asia, Southeast Asia, Northeast India, and North Australia. The species has uniquely shaped seeds resembling a mantis head, which is also uncommon within the same genus *Trichosanthes*. The seed has three chambers: one in the middle where the embryo is positioned – named middle chamber, and one on each side, which are filled with white fibrous material – named side chambers. Despite its uniqueness, the function and biological meaning of this shape remains unknown. This project aims to understand the biological meaning and adaptation underlying this shape. I hypothesize that seed's shape serves to enhance water absorption but also to retain water during the susceptible germination phase. This could possibly have evolved in response to past tropical monsoon climates, characterized by alternating dry and wet seasons. To explore the hypothesis, two sub-hypotheses were tested experimentally. The first sub-hypothesis examined the difference in water absorption and dehydration curve of *T. pilosa seeds* with sealed side chambers compared to untreated *T. pilosa seeds* as a control. The results showed that there is a significant difference in water bearing capacity between control seeds and side chamber-sealed seeds. This indicates the influence of side chambers on the seed's water absorption capacity. The second sub-hypothesis examined germination rates of seeds after different treatments - control seeds with no treatments, seeds that are treated with sulfuric acid, seeds with sealed side chambers with wax, and seeds treated with both wax and sulfuric acid. The statistical analysis revealed no significant differences in germination rates among various treatments, except for a slightly notable distinction between control seeds and those treated with wax and sulfuric acid. However, these statistical outcomes could be attributed to error, potentially arising from the low germination rate.

Sammanfattning

Trichosanthes pilosa (*T. pilosa*), även känd som Japanese snake gourd, är en av växterna i familjen Cucurbitaceae vilka är anpassade för att växa i tropisk monsun, vått tropiskt och fuktigt subtropiskt klimat i Ostasien, Sydostasien, nordöstra Indien och norra Australien. Till skillnad från övriga arter inom släktet Trichosanthes har fröerna en speciell form som liknar huvudet på en bönsyrsa. Frön består av tre kamrar- en i mitten där embryo existerar- namngiven central kammare, och en på varje sida vilka är fyllda med vit fiber-liknande material- namngiven sidkammare. Trots denna påtagliga skillnad är funktionen hos och den biologiska betydelsen av denna form okända. Detta projekt syftar till att förstå den biologiska betydelsen och anpassningen som resulterade i denna form. En hypotes är att formen bidrar till att förbättra upptagning och lagring av vatten under groddfasen som är känslig för miljöförändringar. Detta är sannolikt ett resultat av tropiska monsunklimat, vilka kännetecknas av omväxlande torra och våta perioder. Hypotesen delades in i två delhypoteser vilka testades med experiment: 1) skillnad i specifikt vattenupptag och 2) dehydratiseringsförlopp mellan T. pilosa-frön med förseglade sidkamrar* och obehandlade frön, vilka utgjorde en kontrollgrupp. Resultaten visade en signifikant skillnad i vattenlagringsförmåga mellan kontrollfrön och frön med förseglade sidkamrar. Detta visar att sidkamrarna påverkar fröets vattenlagringsförmåga. Vidare undersöktes grobarheten hos behandlade frön och jämfördes med –obehandlade kontrollfrön. Behandlingen bestod av svavelsyra, vaxförsegling av sidkamrarna samt både svavelsyra och vaxförsegling. Den statistiska analysen visade inga signifikanta skillnader i grobarhet mellan olika behandlingar förutom en svagt märkbar skillnad mellan kontrollfröna och frön som behandlats med vax och svavelsyra. Denna skillnad kan dock tillskrivas fel som potentiellt uppstår på grund av den låga grobarheten.

Background

Trichosanthes. pilosa (=T. pilosa) is in the plant family *Cucurbitaceae*. These plants are adapted to grow in tropical monsoon climate, wet tropical climate, and humid subtropical climate.[1] The species is distributed in Japan, North Australia, Southeast Asia (Borneo, Hainan, Jawa, lesser Sunda islands, Peninsular Malaysia, Maluku, Myanmar, New Guinea, Philippines, Sulawesi, Sumatera, Thailand, Vietnam) and Northeast India[2][3]. *T. pilosa often* grows at the edge of a wood in a hilly area. They have white lacelike flowers with long-fringed petals (Figure 1). Fruits are red when ripe and have a spheric or ellipsoidal shape, with a diameter varying from 3 to > 8 cm (Figure 2,3). Fruits are fleshy when fresh, and seeds are dispersed by birds. The number of the seeds varies depending on the fruit size but usually, there are about 20-30 seeds per fruit. The species has uniquely shaped seeds that looks like a mantis head (Figure 4), which is also uncommon in in plants, even within the genus, *Trichosanthes*, and its function of this shape is unknown. The seed has three chambers: one in the middle where the embryo is positioned – named middle chamber, and one on each side, which are filled with white fibrous material – named side chambers (Figure.4). The width of the seed is around 1 cm. The purpose of this project is to understand the biological meaning of the seed's shape.

Figure 1

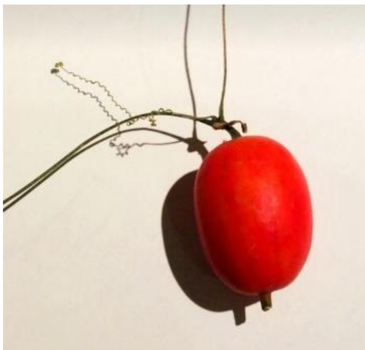

Figure 3

Figure 2

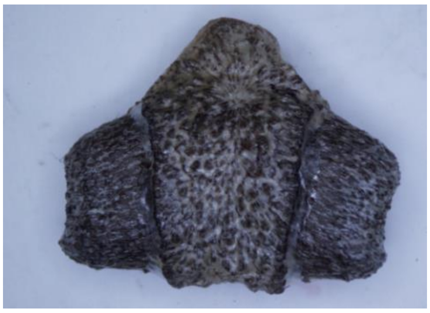

Figure 4

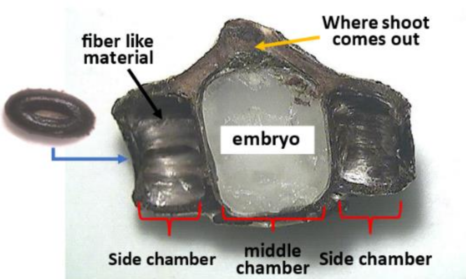

Figure 5
Structure inside Trichosanthes pilosa seed

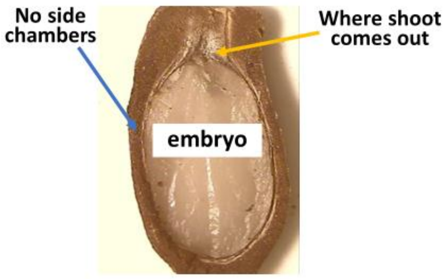

Figure 6
Structure inside the seed of closely related species, Trichosanthes *kirilowii*

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[1]Hugo, de Boer., Karin, Steffen., Wendy. E, Cooper. Sunda to Sahul dispersals in *Trichosanthes* (Cucurbitaceae): a dated phylogeny reveals five independent dispersal events to Australia. Journal of Biogeography. Vol 42,2015: 519-531. doi: 10.1111/jbi.12432

[2] ibid

[3]Royal Botanic Gardens Kew, *Plants of the World Online,* Trichosanthes pilosa. https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:294274-1#distributions, (accessed 30 August 2023)

Former conducted experiments and initial hypothesis

Based on comparison and observation of *T. pilosa seeds* with other seeds of closely related species in the genus Trichosanthes –particularly *Trichosanthes. kirilowii*, it was confirmed that side chambers are the special characteristic structures that make the *T. pilosa* seed's shape unique. This observation led to the hypothesis that the side chambers of *T. pilosa seeds* play a crucial role in water absorption and storage that possibly promote germination. To test this hypothesis, a germination experiment was conducted comparing germination rates of controlled seeds and seeds with eliminated side chambers. Additionally, another experiment was conducted to assess whether the wall of the middle chamber permits water to pass from the side chambers. This was accomplished by applying red dyed water into the side chambers and verifying whether the walls facing middle chamber turned red. In the former experiment, although sample size was limited, seeds without side chambers tended to have a delayed germination as compared to controlled seeds. The results of the latter experiment indicated that the middle chamber's wall does allow water transfer from the side chambers. These findings suggest that the shape of the seed indicates its capacity for water absorption and retention, which potentially contributes to the germination process.

The Influence of paleoclimate on the seed's shape

T. pilosa seed shape, that possibly facilitates absorption and storage of water, may not be as beneficial in humid subtropical climate or wet tropical climate, where availability of water is not a problem for germination due to the high precipitation in these regions. According to Köppen climate classification, wet tropical climates (Af) are characterized by 180-250 cm of precipitation in a year[4]. The minimum is 6 cm of precipitation in the driest month in these climates[5]. Also, it is known that in Af, there are no seasonal changes in terms of thermal and moisture changes[6]. Humid subtropical climate (Cfa/Cwa) is characterised by evenly distributed precipitation throughout year, with total precipitation in a range of 70-200 cm.[7] On the other hand, tropical monsoon climate (Am) differs from Af and Cfa/Cwa, in terms of seasonal change of precipitation.[8] The annual rainfall range is 200-250 cm[9]. Also, Am climates have less than 60 mm of precipitation in the driest month.[10] Therefore, a new hypothesis was made that this unique seed shape possibly evolved in tropical monsoon climates in the past, where both a dry and a rain period exists, with a need for stored water for seed germination. From previous study, it is estimated that *Cucmeroides* section including *T. pilosa* diverged from the most closely

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[4]SKYbrary, Tropical wet climate (Af). https://skybrary.aero/articles/tropical-wet-climate-af , (accessed 30 August 2023)

[5] Encyclopedia Britannica.marine west coast climate.Encyclopedia Britannica, 2023.https://www.britannica.com/science/marine-west-coast-climate. (Accessed 30 August 2023)

[6] SKYbrary, Tropical wet climate (Af). https://skybrary.aero/articles/tropical-wet-climate-af , (accessed 30 August 2023)

[7] Encyclopedia Britannica.humid subtropical climate. Encyclopedia Britannica, 2023.https://www.britannica.com/science/humid-subtropical-climate. (Accessed 30 August 2023)

[8] SKYbrary, Tropical monsoon climate (Am). https://www.skybrary.aero/articles/tropical-monsoon-climate-am , (accessed 30 August 2023)

[9]PMFIAS, "Monsoon Climate/Monsoon Forests",2018. https://www.pmfias.com/tropical-monsoon-climate-tropical-marine-climate-tropical-monsoon-forests/, (accessed 30 August 2023)

[10] Encyclopedia Britannica.marine west coast climate.Encyclopedia Britannica, 2023.https://www.britannica.com/science/marine-west-coast-climate. (Accessed 30 August 2023)

related section *Trichosanthes* around 25 M years ago in mainland Asia[11]. Also, it is estimated that the common ancestor of species in *Cucumeroides* section originated in mainland Asia and Malay Archipelago[12]. It is known that species in the *Cucmeroides* section have seeds with side chambers, which is only found in this section[13]. According to research estimating origin of Asian and Australian monsoons, a result of a simulation showed that Southeast Asian monsoon existed in late Oligocene (25M years ago) and that it expanded toward Northeast Asia till late Miocene (10M years ago)[14]. This can be a support to the hypothesis that the seed shape of *T. pilosa* and Cucumeroides section species evolved in the tropical monsoon region, taking into consideration time and space species existed according to the biogeographic analysis.

Seed dormancy

Seed dormancy is another important element in explaining and understanding the evolutionary mechanisms behind seed viability and germination. Seed dormancy is an adaptation in seeds that allows for survival under various conditions, for instance to tolerate heat and dryness until the rain period begins. When the seeds are in a dormant state, they are not able to germinate[15]. There are different kinds of seed dormancies, such as morphological dormancy, mechanical dormancy, physical dormancy, and combined dormancy – where each relies on different mechanisms[16]. For example, physical dormancy is due to hard seed coats that are not water permeable[17]. The seed coats may also help the seeds to not lose water. To break physical dormancy in natural conditions, seeds need to be exposed to, for instance, rain, high temperatures, or fluctuating diurnal temperatures.[18] Physical dormancy is a mechanism of desiccation avoidance, or not permitting seeds to germinate at inappropriate time, such as in a dry period. On the other hand, physical dormancy may have a demerit, that it cannot keep the same function when the seed coats are damaged. When the seed coats are damaged, physical dormancy enables the seeds to absorb water, which leads to a break in dormancy and germination. However, once the seed coats are damaged, it is also easy for the seeds to lose water, which may be risky for the early, susceptible stage of germination.

I hypothesize that the seed shape of *Trichosanthes. pilosa is* a solution to this problem- the trade-off between ability to absorb water and keep water, possibly by seeds having shapes that are adapted to absorb water but also to keep water during the susceptible germination period.

As one step to understand this complex germination/seed shape adaptation more deeply, a germination experiment was previously conducted to determine the seed dormancy type of *Trichosanthes pilosa*. It is known that physical dormancy could been broken by acid scarification. Although sample size was limited, the results revealed that sulfuric acid-treated seeds exhibited a 40% germination rate, whereas untreated control seeds only showed 10% germination. This

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[11] Hugo, de Boer., Karin, Steffen., Wendy. E, Cooper. Sunda to Sahul dispersals in *Trichosanthes* (Cucurbitaceae): a dated phylogeny reveals five independent dispersal events to Australia. Journal of Biogeography. Vol 42,2015: 519-531. doi: [10.1111/jbi.12432](10.1111/jbi.12432)

[12] ibid

[13] Thulin, Mats; professor at Uppsala University of systematic biology and organism's biology. Interview 2018-08-15.

[14] Xiaodong, Liu., et.al. Continental drift and plateau uplift control origination and evolution of Asian and Australian monsoons. Scientific Reports Vol 7, 2017:3. doi:10.1038/srep40344

[15]Oregon State University, GERMINATION: SEED DORMANCY. https://agsci.oregonstate.edu/mes/sustainable-wildflower-seed-production/germination-seed-dormancy, (accessed 30 August 2023)

[16] Carol.C, Baskin., and Jerry.M, Baskin. Seeds. Second edition. Academic Press, 2014.

[17] Ibid

[18] Ibid

implied that *T. pilosa seeds* possibly have physical dormancy. However, the result from another experiment implied the contrary, that seeds of *T. pilosa do* not have physical dormancy. An experiment examining change in seed embryo moisture content was conducted. After 15 hours of water immersion, the embryos displayed an average moisture content of 18.34% across 50 seeds. The results implied that seeds of *T. pilosa do* not have physical dormancy, as the embryo could absorb water.

Thus, further replicated experiments are needed to explore potential connections between acid scarification and other forms of seed dormancy. Baskin and Baskin (2014) [19] stated that the dormancy type of this plant's seed is inferred to be physiological dormancy, yet further investigation is needed to validate this inference.

Summary and hypotheses explored in this study

In summary, I hypothesize that *Trichosanthes. pilosa'* s seed's shape serves to enhance water absorption but also to retain water during the susceptible germination phase, which could possibly have evolved in response to past tropical monsoon climates, characterized by alternating dry and wet seasons. Seed dormancy is also an important factor to understand complex germination/seed shape adaptation, even if it is not related to the hypothesis of the present study. I hypothesize that *T. pilosa seeds* have physiological dormancy or possibly mechanical dormancy. Mechanical dormancy is characterized by inability of seeds to swell and for the radicle o begin grow due to the presence of a hard mechanical barrier.[20]

Several sub-hypotheses were tested in this project that potentially support the main hypothesis.

To test the sub-hypothesis- "*T. pilosa seeds* have a shape that can both absorb and store water", a comparison was made between the imbibition and dehydration curves of control seeds and seeds with sealed side chambers[21].

To test the sub-hypothesises- "acid scarification of *T. pilosa* seeds removes certain forms of seed dormancy" and "*T. pilosa seeds* with sealed side chambers have lower germination rate potentially due to reduced water absorption and retention capabilities," a germination experiment on *T. pilosa* seeds with different treatments was conducted.

Materials and methods

Materials

Seeds of *Trichosanthes. pilosa* used in this project was collected in Chiba, Japan in Autumn 2021.

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[19]Ibid

[20]Nigel.J, Slator., Andrew.N, Callister., & Nichols. J, Doland. Mechanical but not physical dormancy is a cause of poor germination in teak (*Tectona grandis* L.f.). *New Forests* Vol 44.2013:39–49. doi.org/10.1007/s11056-011-9298-0

[21]Jannathan, Mamut., Dun-Yan, Tan., Carol.C, Baskin., *et al.* Role of trichomes and pericarp in the seed biology of the desert annual *Lachnoloma lehmannii* (Brassicaceae). *Ecol Res* Vol 29, 2014:33–44. doi: 10.1007s11284-013-1098-x

Finding the seed's imbibition and dehydration curve

The aim of this experiment is to test the statistical hypothesis that "control *Trichosanthes. pilosa seeds* exhibit significantly superior water absorption and retention compared to *T. pilosa* seeds with wax-sealed side chambers.

Preparing materials

Each experimental group consisted of 54 *T. pilosa* seeds. Seeds subjected to the wax treatment were infused with molten candle wax within their side chambers, using a disposable syringe. To prevent wax solidification within the syringe, it was cleaned with hot water between injections.

Monitoring moisture content dynamics

The initial dry weight of both the control seeds and the wax-sealed seeds was recorded. Subsequently, either of the seed groups was placed within a water-filled petri dish, which was then covered with its lid. A timer was set for a 30-minute interval. After 15 minutes, the same procedure was executed for the alternate seed group. Following a 30-minute immersion period, the seeds were removed from the water and placed on a strainer to eliminate remaining water. Seed weight was measured using an electronic balance, and the seeds were returned to the water-filled petri dish after weighing. This process was repeated over a span of approximately 6 hours. After the imbibition phase, the monitoring shifted to dehydration. Similar protocols were applied for dehydration monitoring, wherein seeds were removed from water, and their weight was recorded under dry laboratory conditions every 30 minutes. Moisture content was calculated using the formula: $(w - d) / w * 100$, where 'w' = wet weight and 'd' = initial dry weight. The same experiment was replicated four times. Absorption and dehydration processes were compared using the same volume of water and the same number of seeds.

Data Analysis

To analyse differences in moisture content between the control seeds and seeds with sealed side chambers, a Generalized Additive Model (GAM) was used in RStudio version 4.3.1 for Windows.

Germination experiment

In this experiment, the following statistical hypothesis was tested: "There is a significant difference in germination rate between control *Trichosanthes. pilosa seeds* and *T. pilosa* seeds with different treatments".

To investigate this hypothesis, four distinct experimental seed groups were established: the control group, wax-treated group, sulfuric acid-treated group, and wax-sulfuric acid-treated group. The control group and wax-treated group were compared to assess the effect of sealing side chambers with wax on germination, which potentially relates to reduced water-retaining ability. The comparison between the control group and sulfuric acid-treated group aimed to check the existence of any kind of seed dormancy. Furthermore, interaction between wax and sulfuric acid treatments were investigated by comparing the control group with the wax-sulfuric acid-treated group.

Preparing materials

A total of 756 *T. pilosa seeds* retrieved from various fruits were used in this study. Each experimental seed group consisted of 130 seeds, except for the wax-sulfuric acid-treated group, which contained 126 seeds. The wax-treated group had their side chambers sealed using the method previously described in the experiment of seed's imbibition and dehydration curve. Seeds of the sulfuric acid-treated group were placed in a petri dish containing a thin layer of liquid sulfuric acid with a concentration of 90% for totally 6 minutes—3 minutes on each side. After sulfuric acid treatment, the seeds were thoroughly washed with running water.

Germination Conditions

Each flowerpot was sown with two to three seeds and labelled with plant markers indicating the experimental seed group and number within the experimental seed group. To prevent biased outcomes, the placements of flowerpots were randomized within each plant tray. The germination environment was maintained in an indoor plant nursery, with a light dark cycle of 12:12h and ambient laboratory temperature. Flowerpots were regularly watered every other day, and germination progress was monitored.

Data analysis

The final germination rate was analysed using a binomial logistic regression in RStudio version 4.3.1 for Windows.

Results

Experiment- seed's imbibition and dehydration curve

All four replicated experiments indicated that control *Trichosanthes. pilosa* seeds exhibited a higher percentage of moisture content in comparison to *T. pilosa* seeds with side chambers sealed using wax. These significant outcomes are visualized through the figures depicting the results of General Additive Model (GAM) analysis of the imbibition and dehydration curve for these seeds (figure7-10).

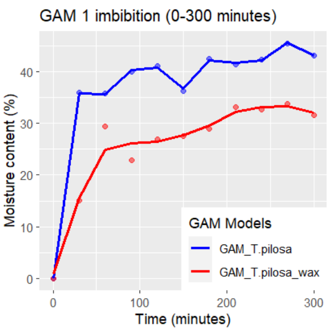

Figure 7a

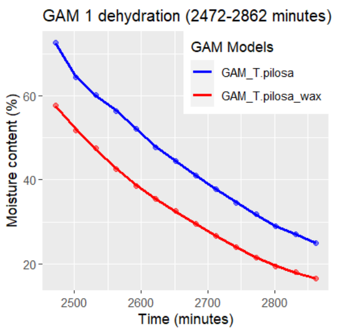

Figure 7b

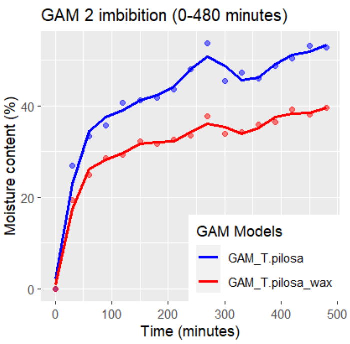

Figure 8a

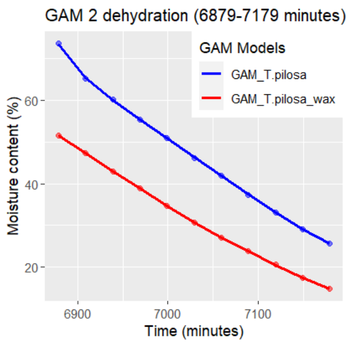

Figure 8b

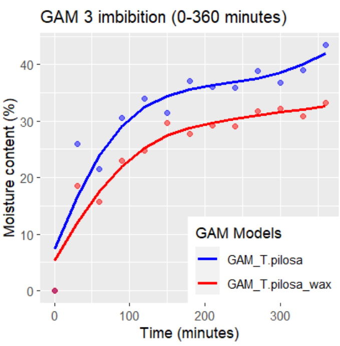

Figure 9a

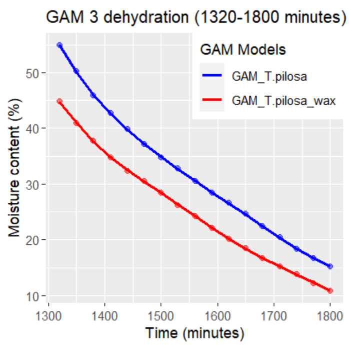

Figure 9b

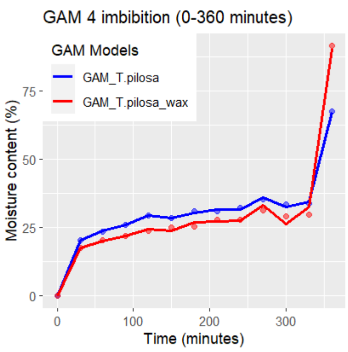

Figure 10a

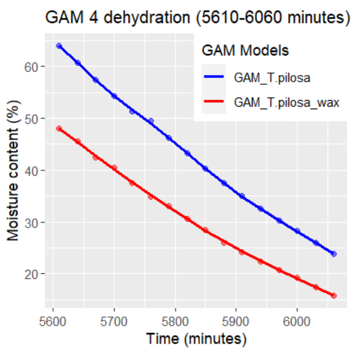

Figure 10b

Figure 7-10: Each set of figures (7a-b, 8a-b, 9a-b, 10a- b) represent the four replicates of the experiment examining seed's imbibition and dehydration curve. In each figure, figure a show the imbibition and figure b show the dehydration curve.

Experiment- seed dormancy and germination experiment

Each of the experimental seed groups exhibited notably low final germination rates: the control group at 10.77%, the wax-treated group at 10%, the sulfuric acid-treated group at 6.92%, and the wax-sulfuric acid-treated group at 3.17% (figure 11).The outcomes of the binomial logistic regression analysis revealed that there were no statistically significant differences in the germination rates between the control and wax-treated groups ($p = 0.839$) or between the control and sulfuric acid-treated groups ($p = 0.278$). However, a slightly significant difference was observed between the control group and the wax-sulfuric acid-treated group ($p = 0.025$)

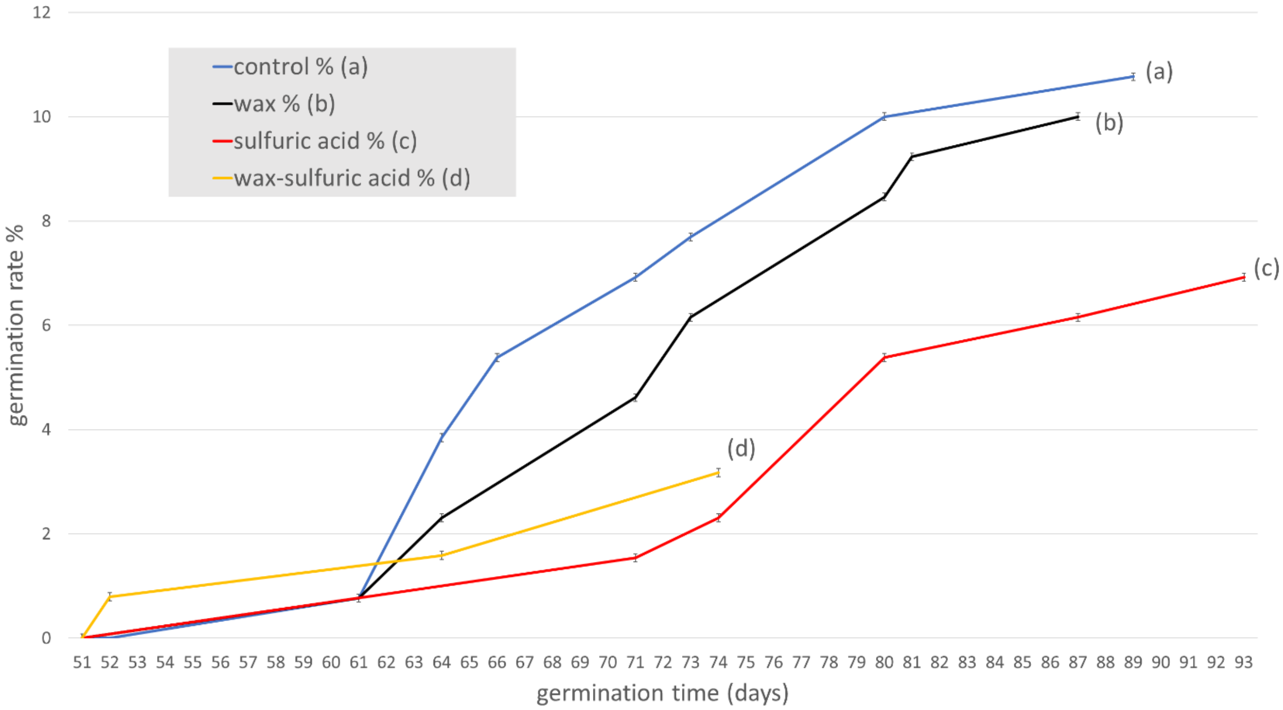

Figure 11 Polygonal line graphs showing germination rate depending on germination time in days. Control group-(a) wax-treated group-(b) sulfuric acid-treated group-(c) wax-sulfuric acid-treated group-(d). Error bars are ±1 SE.

Discussions

Experiment- seed's imbibition and dehydration curve

The experiment's outcomes revealed a notable distinction in the water-bearing capacity between control seeds and seeds with sealed side chambers. These findings suggest that side chambers play a crucial role in water storage, potentially contributing to the germination process.

Experiment- seed dormancy and germination experiment

The statistical analysis revealed no significant differences in germination rates among various treatments, except for a slightly notable distinction between control seeds and those treated with wax and sulfuric acid. However, I suspect these statistical outcomes could be attributed to error, potentially arising from the low germination rate observed. The low germination rate is possibly explained by unfavourable germination conditions, especially the laboratory temperature that did not match the required conditions for this plant's natural habitat. Unfortunately, controlling temperature was unfeasible in this experiment, compelling the germination process to occur under ambient laboratory conditions. Consequently, drawing definite conclusions regarding the proposed hypotheses becomes challenging. The aims to understand the impact of wax-sealed side chambers on germination, the existence of seed dormancy, and the interaction between wax and sulfuric acid treatments remain inconclusive.

Improvements

To enhance the germination experiment, favourable conditions could be implemented. Temperature control in a laboratory setting allows for optimal germination temperatures. Furthermore, ensuring uniform and consistent lighting across all flowerpots remains a challenge, although efforts were made to minimize bias by randomizing flowerpot positions. Utilizing more recently collected seeds is another potential improvement to consider. Alternative, less harmful waxes, such as beeswax, could have been used for wax-treatment in germination experiment.

Hypothesis, Conclusion, and Future Research Directions

In summary, the outcomes of the experiments conducted in this project revealed some essential factors that support to the main hypothesis of this study: " *Trichosanthes. pilosa* s seed's shape serves to enhance water absorption but also to retain water during the susceptible germination phase. These traits could possibly have evolved in response to past tropical monsoon climates." The results of the experiment, as illustrated by the seed's imbibition and dehydration curves, provided evidence that side chambers contribute to maintain water, which potentially is advantageous during the germination phase. However, it's important to note that the germination experiment, intended to explore this connection between seed germination and the side chambers' water-retention capacity, could not yield conclusive results due to the low germination rate, likely an effect of unfavourable germination conditions. Hence, conducting additional germination experiments under improved conditions is indispensable to unveil a clearer understanding of the expected correlation between germination and the water-retention capacity of side chambers. For further investigations, the approaches to this main hypothesis fall into following four categories-

1. **In-depth Analysis of *Trichosanthes pilosa* seed morphology traits:**

- Comparison of the imbibition and dehydration curves of *Trichosanthes pilosa* seeds with closely related species such as *T. cucumerina*, *T. kirilowii*, and *T. beccariana*. This comparison could involve assessing imbibition and dehydration based on the same seed mass-to-water volume ratio or utilizing simulations of imbibition and dehydration under consistent seed volume-to-water volume conditions. In the latter case, the 3D scanning of seed structures would be necessary for computer modelling.

-Monitoring water movement within the seed to gain a more comprehensive understanding of the imbibition process. This study would explore fluid dynamics, focusing on how water moves within seeds during imbibition, potentially affected by processes such as capillarity flow or diffusion[22].

2. Germination experiments and seed shape relationship:

- As mentioned above, a replication of the germination experiment conducted in this project with improved germination conditions is needed.

- Germination experiments that enable simultaneous monitoring of moisture content and water flow within the seed during germination could provide valuable insights. Applying this approach to *T. pilosa seeds*, as well as to *T. pilosa* seeds with wax-sealed side chambers and closely related species, could unveil intriguing relationships.

3. Biogeographical and paleoclimatic understanding:

- Integrating paleoclimate investigations with comprehensive distribution analyses, such as herbarium studies and fieldwork assessing the distribution of *T. pilosa and* closely related species within the *Trichosanthes* genus. Particularly focusing on species belonging to the same Cucumeroides section as *T. pilosa,* which are recognized for their possession of side chambers, could yield fascinating insights into the evolution of this trait.

4. Understanding whole germination process

- Further research on seed dormancy in *T. pilosa* seeds is necessary. An experiment to assess physiological dormancy through gibberellin treatment should be conducted.

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