CHAPTER IV

RESULTS and DISCUSSIONS

4.1 Floral morphology and anther dehiscence of *L. aromatica* and *L. geoffrayi*

The floral color of *L. aromatica* is violet. While *L. geoffrayi* has dark violet, purplish pink, or white (Figure 4.1). The color variation of *L. geoffrayi* is firstly reported here, indicating that there are phenotypic forms in a population. It may be assumed that self-pollination promotes the recessive genes (Li, Q., Ruan, C.-J., and Silva, J. A. T. D., 2017), or mutations could naturally occur leading to color variations, for example, pink and white forms among these populations.

The floral shape of *L. aromatica* and *L. geoffrayi* is tubular floral with bilateral symmetry type Willmer, P., 2011), but it is small size (1-1.3 cm long) and slightly bilateral symmetry. The corolla consists of five unequal lobes, two upper lobes and three lower lobes, as indicated in Flora of Thailand (Smitinand, T. and Larsen, K., 1990). From my observation, in the early bud stage, the upper lobes cover the lower lobes (Figure 4.2-A1 and 4.2-B1). However, the upper lobes are positioned on the lower side of natural position when it is fully open. The outer surface of the corolla of both species is hairy, but more distinct in *L. aromatica* (Figure 4.2-A2 and 4.2-B2). The inner surface of the corolla of both species is distinctly and densely hairy from the base of the upper lobes. Trichomes found on one side of the corolla tube (Figure 4.2-A3 and 4.2-B3) and provided were insects with a footing, encouraging them to enter the flower correctly. Indeed, it plays an important role for pollinators. (Kampny, C. M., 1995).

Figure 4.1 The floral colors of *Limnophila aromatica*: A-B. violet corolla. *L. geoffrayi:* C. dark violet, D. purplish pink, and E. white corolla.

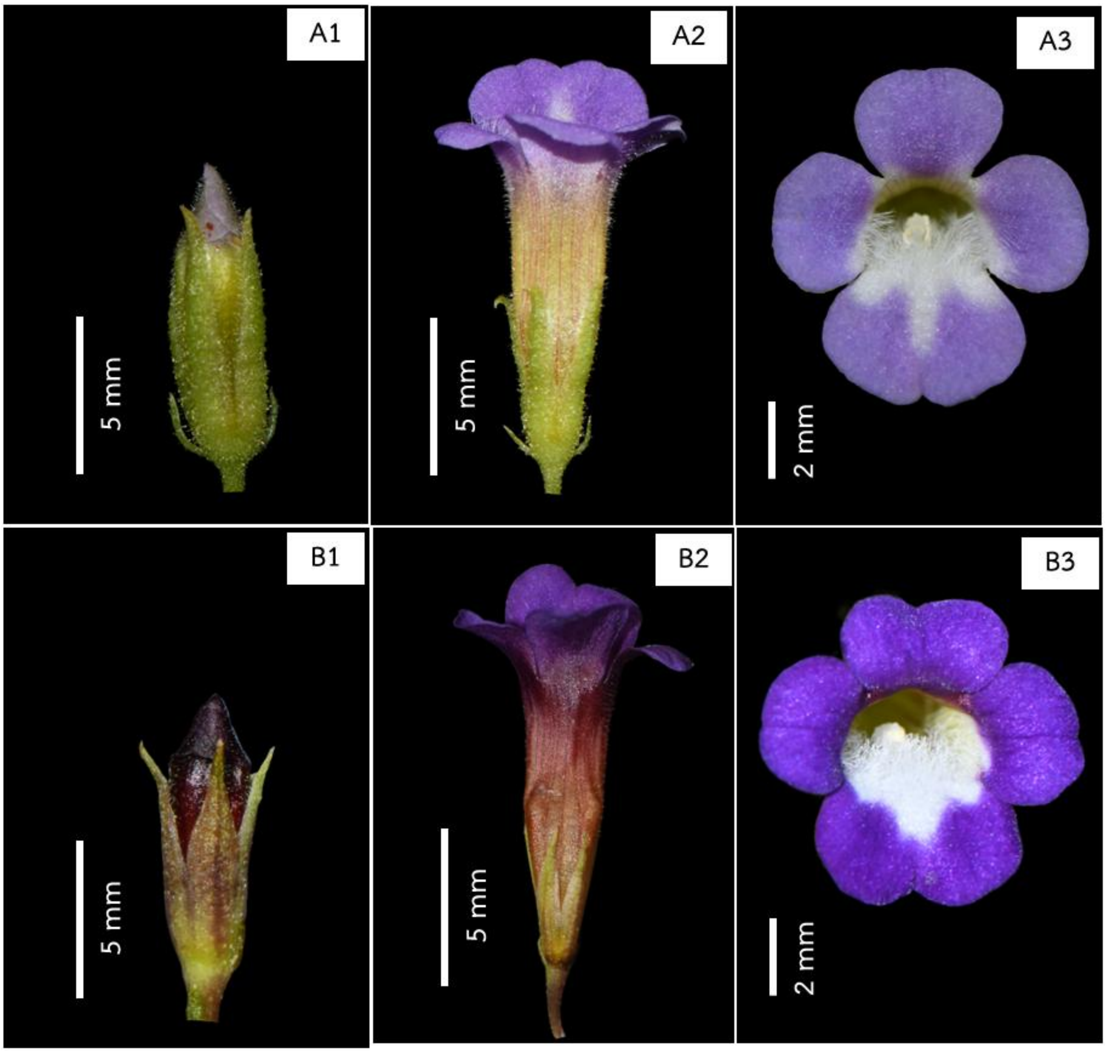

Figure 4.2 Floral morphological comparison of *Limnophila aromatica*: A1. early bud stage, A2. open floral stage, and A3. top view of floral. *L. geoffrayi*: B1. early bud stage, B2. open floral stage, and B3. top view of flower.

Both species have four stamens arranged in two pairs, two short ones located on the surface of the corolla tube at the upper lobe side, and other 2 long ones located on the surface of the corolla tube on the lower lobe side. Characteristically, the four anthers are inserted in the corolla tube and positioned in vertical arrangement inside the corolla tube, rather near to the upper lobe side (Figure 4.3-A1, B1, C1 and 4.4-A1, B1, C1). This may facilitate the insect's claw and step above the four anthers. The position of anthers and stigma in both species can promote self-pollination because the longer stamen pairs are positioned above the stigma. This pattern has been reported in some Scrophulariaceae and some taxa of Plantaginaceae (Kampny, C. M., 1995). Moreover, the shorter pair of the anther is located under the stigma lobe and deeper forward to the base of the corolla tube. Thus, it may induce the insect to go deeply into collecting the pollen, especially pollen collected insects.

The position of stamens and stigma in different floral stages of both species was investigated, as shown in Figure 4.3 and Figure 4.4. I divide flowers into three different stages, which are the early buds (corolla lobes have not yet opened and emerged slightly from the calyx), late buds (corolla lobes have not opened yet but are almost fully developed), and open flowers (corolla tube opens). In the early bud stage, the position of the shorter pair of stamens is lower than the stigma, and the longer pair of stamens is the same length as the stigma. On the other hand, the late bud stage and open flower have an elongated development of stamens, the longer pair of the anther extends and locates above the stigma, while the shorter pair is still below the stigma. Indeed, the position of the stamen and stigma promotes self-pollination in each flower. This characteristic is commonly found in *Limnophila*.

The anther attachment types of both species are dorsifixed. It is ca. 0.13 mm wide and ca. 0.27 mm long. The anther dehiscent in different floral stages of both species was investigated, as shown in Figure 4.3-A2, B2, C2 and Figure 4.4-A2, B2, C2. In the early bud and the late bud stages, the anther wall surface is smooth and not split. While in the open floral stage, the anther wall surface is rough and breaks. The anther dehiscence type is longitudinal, which is the most common type and a primitive characteristic of the anther dehiscence in the angiosperms. Normally, the anther will separate along a suture that runs parallel to the long axis of the thecae (Simpson, M.

G., 2019). I assumed that the heat from sunlight provides drier air around the anther and makes them break since the anthers were broken in the open floral stage. This study did not examine stigma receptivity, whether the stigma is ready for pollination before the anther disperse. However, in the late bud stage, the stigma surface of both species presented the stigma surface and was not ready for facilitating the pollen germination (Zulkarnain, Z., Eliyanti, E., and Swari, E. I., 2019). The active surface of the stigma can be recognized by having a sticky exudate on its surface (Wu Hai et al., 2008). Both species of *Limnophila*, the surface of the stigma is covered by a sticky exudate showing ready for pollination (Figure 4.3-C1, 4.4-C1) and facilitating the germination of the pollen.

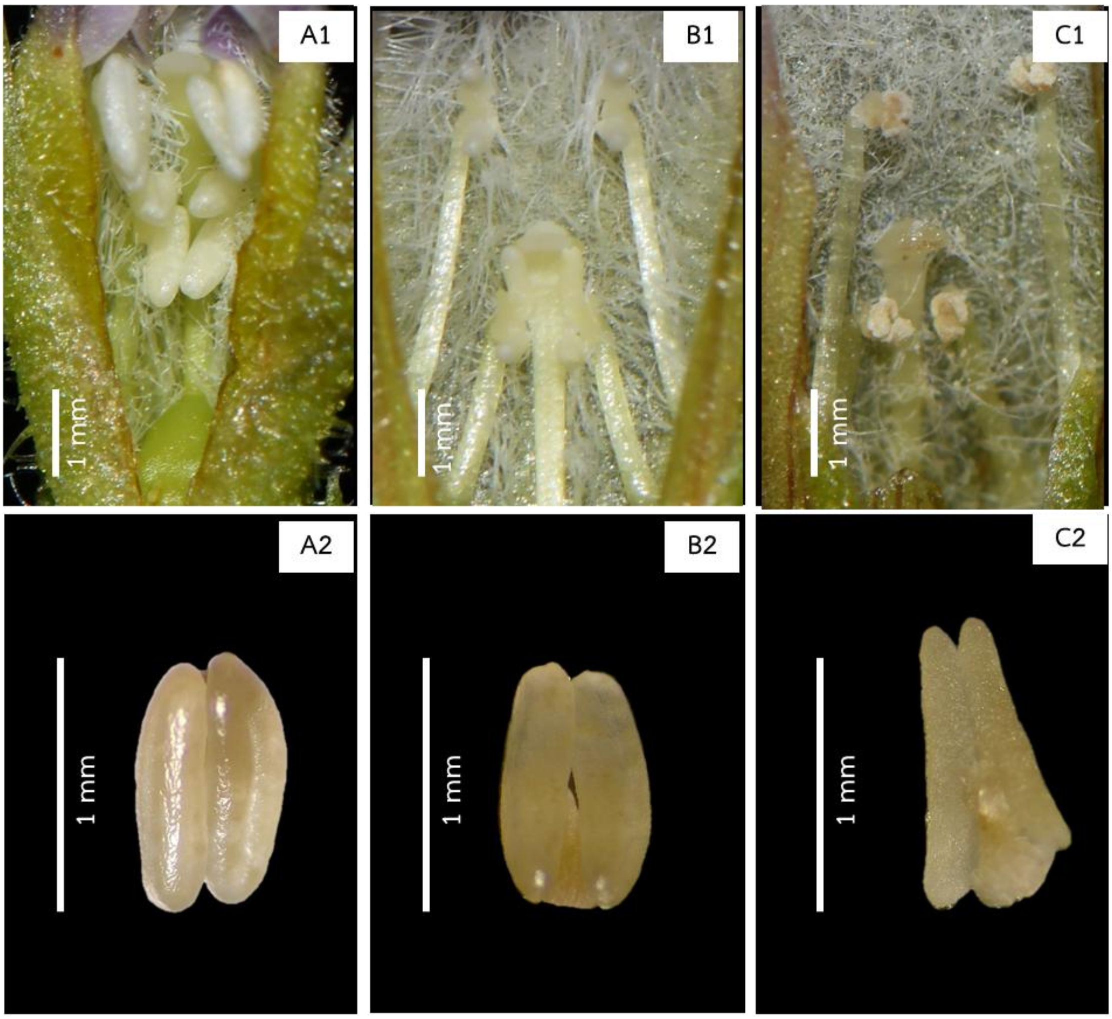

Figure 4.3 Position of sex organs and anther dehiscence at different floral stages of *Limnophila aromatica*. A1-A2: Early bud, B1-B2: Late bud, and C1-C2: Open floral stage.

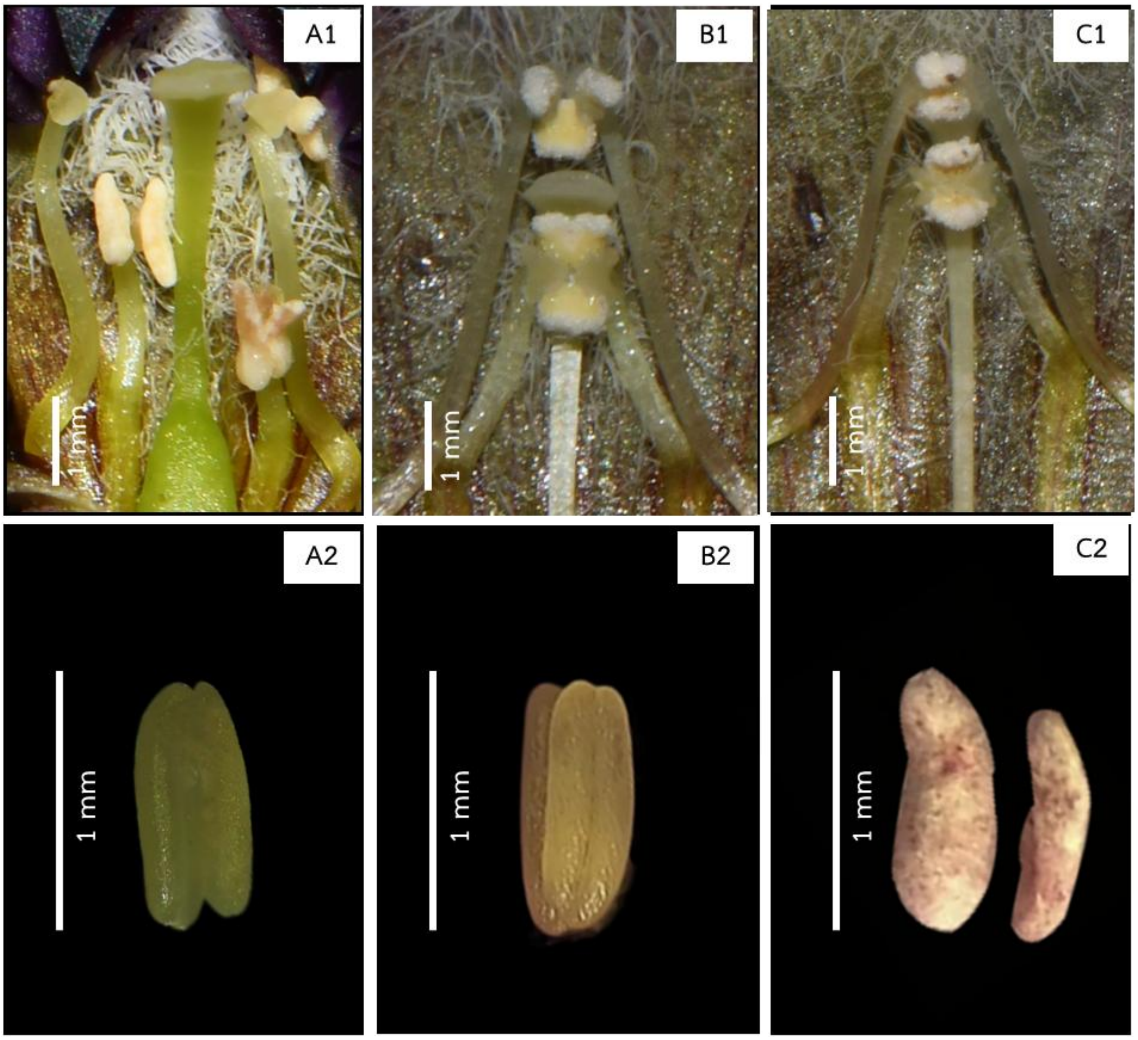

Figure 4.4 Position of sex organs and anther dehiscence at different floral stages of *Limnophila geoffrayi*. A1-A2: Early bud, B1-B2: Late bud, and C1-C2: Open floral.

4.2 Floral phenology of *L. aromatica* and *L. geoffrayi*

The individual floral opening period of *L. aromatica* and *L. geoffrayi* florals by tracking late-stage buds, it was found that *L. aromatica* florals opened only one day at daytime (6.31±0.66 hours; N = 48). The flowers started to open around 10:00-11:00 am, and fully opened 100% at 01:00-02:00 pm, then closed around 5:00-6:00 pm nearly sunset and fallen off the next day (Figures 4.5 and 4.6-A). On the other hand, some flowers of *L. geoffrayi* (about 1/3 of total flowers) opened 2 days (4.88 ± 1.07 hours on day one and 1.53 ± 2.35 hours on day two; N = 43). The first day, flowers started to open at 10:00-11:00 am, fully opened 100% around 01:00-02:00 pm, and close at 05:00-06:00 pm, and opened again at 10:00-11:00 am on the second day, fully opened only 32% of all flowers at 01:00-02:00 pm then closed around 05:00-06:00 pm and fallen off the next day (Figures 4.5 and 4.6-B). However, the open flowers in the studied population presented about one month.

This is the first report on the floral opening pattern of *Limnophila*. The floral opening patterns depended on species. The longer individual floral blooming pattern, more than one day of *L. geoffrayi* than *L. aromatica* may increase the chance of insects entering the flower. This pattern is similar to *Gossypium turneri*, which blooms for 2 days (Yescas-Romo, K. F., Hayano-Kanashiro, C., and Molina-Freaner, F., 2024).

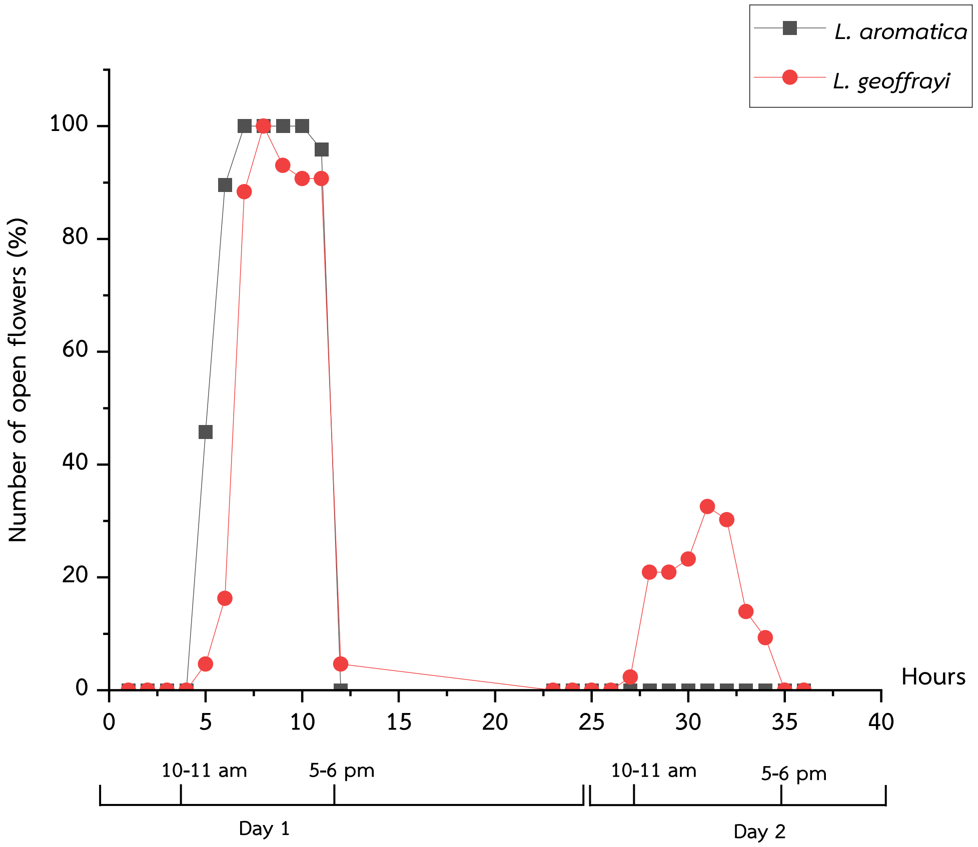

Figure 4.5 Percentage of open flowers of *Limnophila aromatica* and *L. geoffrayi* in the floral opening period.

Figure 4.6 Flower opening patterns of both species during the floral opening period. A: *Limnophila aromatica*, and B: *L. geoffrayi*.

4.3 Breeding systems

The result of breeding systems by bagged experiment to avoid pollinators of both species of *Limnophila* is shown in Table 4.1. Comparisons among species and breeding system types to fruit set were significantly different by GLM ($p<0.001$). *L. aromatica* showed no fruit set in any of the treatments (Figure 4.7), suggesting that the species may has completed self-incompatibility. The individual plant derived by cutting and came from the same genetic clone revealed no fruit setting, confirming the self-incompatibility. I assumed that the genetic diversity of this species is low. This species has not been found in a natural population in Thailand. However, it is quite interesting to breed different clones to increase the genetic diversity of this crop plant and check whether it could produce seed or not. In contrast, *L. geoffrayi*, has a 100% fruit set on autogamy and natural pollination (Figure 4.8), indicating self-compatibility. Neither of the species had parthenogenesis occurred because emasculation treatment did not set fruit (Table 4.1). For genetic diversity based on breeding system patterns, showing self-pollination species has rather low genetic diversity compared with cross-pollination species (Zhang, D., Li, Y.-Y., Zhao, X., Zhang, C., Liu, D.-K., Lan, S., Yin, W., and Liu Z.-J., 2024). From a conservation point of view, seed banking from different populations is a crucial need for keeping their genetic diversity.

Table 4.1 *Limnophila aromatica and L. geoffrayi* 's fruit set and treatments for pollination (Fruit set percentages with different letters are significantly different using Dunn-Sidak test at $P<0.05$).

Species	Pollination treatments	Number of florals	Number of fruits	Fruit set (%)
L. aromatica	Autogamy	30	0	0^{b}
	Emasculation	30	0	0^{b}
	Natural pollination	30	0	0^{b}
L. geoffrayi	Autogamy	30	30	100^{a}
	Emasculation	30	0	0^{b}
	Natural pollination	30	30	100^{a}

Figure 4.7 *Limnophila aromatica* 's fruit set and treatments for pollination. A1-A3: Autogamy, B1-B3: Emasculation, and C1-C3: Natural pollination. (Scale bar = 5 mm.)

Figure 4.8 *Limnophila geoffrayi* 's fruit set and treatments for pollination. A1-A3: Autogamy, B1-B3: Emasculation, and C1-C3: Natural pollination. (Scale bar = 5 mm.)

4.4 Pollination

A total of 84 individual insects were observed to visit the flowers of *L. aromatica,* whereas 60 individual insects visited the flowers of *L. geoffrayi*. The main visitors of both species were solitary bees belonging to the Hymenoptera in various orders; furthermore, other orders, such as Coleoptera and Thysanoptera, were rarely seen and did not exhibit pollen-collecting behavior, only visiting flowers (Table 4.2). The visitors of both species were similar at 0.5 based on Sorensen’s similarity index, which were *Braunsapis puangensis*, *Lasioglossum* (*Ctenonomia*) *albescens*, (*Ctenonomia*) *vagans* gr., and *Thrips* sp. This study revealed that the number of flower visits on *L. aromatica* in the cultivated plot was higher than that of *L. geoffrayi* in the natural habitat. This may be due to the greater diversity of flowering plants in the natural habitat where *L.*

geoffrayi was located (Figure 4.9). The main visitors of both *Limnophila* species were pollen-collecting bees. I observed that they collected the pollens from other flowering plant species, such as *Ipomoea aquatica*, *Lobelia thorelii*, *Murdannia* sp., and *Xyris* sp. in the natural habitat of *L. geoffrayi* (Figure 4.9). Indeed, *Limnophila* flower served as insect food. There are few reports on the pollination of *Limnophila.* Kato, M., Kosaka, Y., Kawakita, A., Okuyama, Y., Kobayashi, C., Phimminith, T., and Thongphan, D. (2008) recorded that the flower visitors of *L. geoffrayi* were small bees*, Lasioglossum* sp., and Laha, S., Chatterjee, S., Das, A., Smith, B., and Basu, P. (2020) recorded that the flower visitors of *L. repens* were bees belonging to various families in Apidae, Halictidae, and Magachilidae.

The visiting time of the main visitors of both species, which are solitary bees, was from 9:00 a.m. to 04:00 p.m. The average temperature was 31 °C, and the average humidity was 64 percent. Most solitary bees enter the flowers at 11:00 a.m.-12:00 p.m. (Figure 4.10), which coincides with the time when the flowers are in full bloom (Figure 4.6). There is another study of the foraging behavior of solitary bees, with the peak time of 10:00-11:00 a.m. and a decline in the afternoon (Lim, Z., Lord, J., and Johnson, S., 2025; Gonzalez, V. H., Mantilla, B., and Palacios, E., 2006). The solitary bees that visited the flowers of both *Limnophila* have landed on the upper lobe of the corolla and clawed into the corolla tube, stepping on the anthers and stigma. The lower pair of stamens make the insect claw deeper into the corolla tube (Figure 4.11 and Figure 4.12). Generally, the time visiting of each insect to each flower was 3-12 seconds, (10.76 ± 11.36). It is impossible to observe the pollination process inside the small corolla tube of both species. However, all the pollen-collecting solitary bees that visited the flower usually have several pollen grains collected in the scopa (an anatomical structure of a bee specifically adapted for the storage and delivery of pollen). Some pollen might be attached to solitary bee legs before it claws to the corolla. The collecting pollen behavior of the solitary bees as described may increase the chance of cross-pollination of the *L. geoffrayi*, since the insect had carried pollen from the previous plants. However, *L. geoffrayi* exhibited a breeding system from self-pollination without vectors (see above). I assumed that the autogamy in *L. geoffrayi* resulted from pollinators scarcity.

Table 4.2 Insect visits of *Limnophila aromatica* (Botanical Garden) compared with *L. geoffrayi* (Rice fields).

Orders	**Families**	**Species**	***L. aromatica***	***L. geoffrayi***
Coleoptera	Coccinellidae	*Micraspis* sp.		x
	Chrysomelidae	*Monolepta* sp.		x
Hymenoptera	Andrenidae	Unknown		x
	Apidae	*Ceratina* (*Neoceratina*) *dentipes*		x
		Ceratina (*Pithitis*) *smaragdula*		x
		Braunsapis hewitti	x	
		Braunsapis malliki	x	
		*Braunsapis puangensis**	x	x
	Halictidae	*Ceylalictus* sp.		
		Lasioglossum (*Ctenonomia*) *albescens* *	x	x
		Lasioglossum (*Ctenonomia*) *vagans* gr.*	x	x
	Magachilidae	*Heriades* (*Michenerella*) sp.		x
Thysanoptera	Thripidae	*Thrips* sp.*	x	x

* Means the species found in both *Limnophila* florals.

Figure 4.9 Some of flowering plants in the study plots of *Limnophila geoffrayi* and insects that forage in the flowers: A. *Ipomoea aquatica*, B. *Lobelia thorelii*, C. *Murdannia* sp., and D. *Xyris* sp.

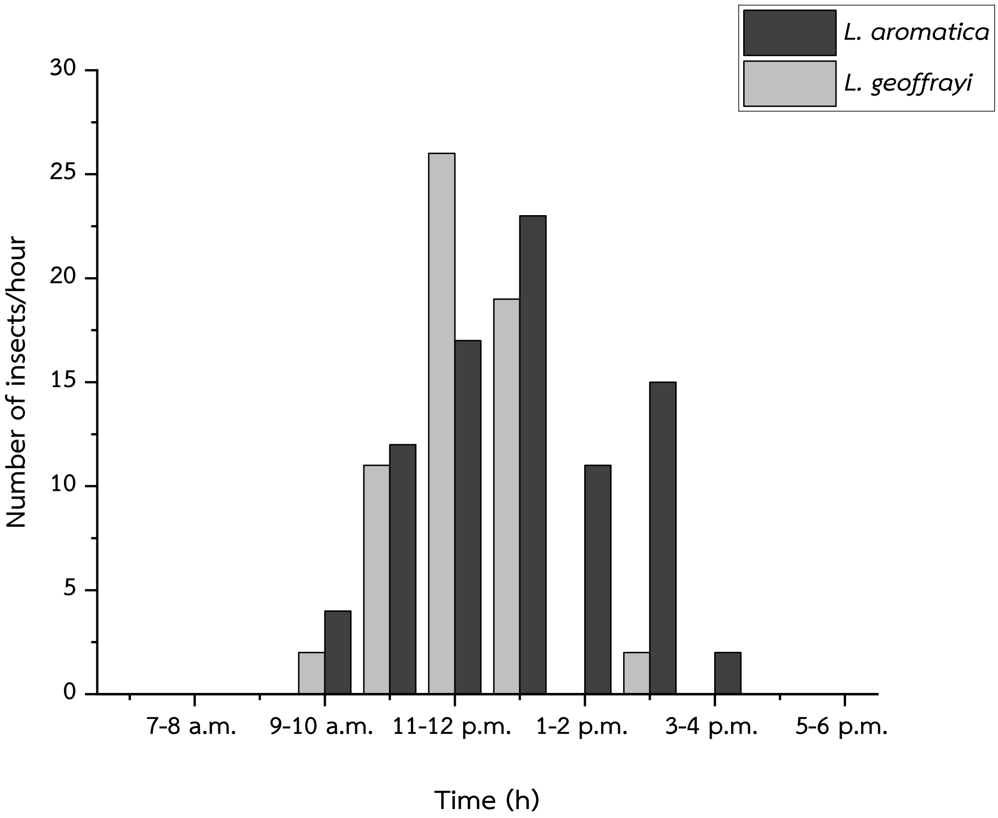

Figure 4.10 Number of insects visiting the flowers of *Limnophila aromatica* and *L. geoffrayi* from 06:00 a.m. to 06:00 p.m., for 3 days (36 h) of the flowering season.

Figure 4.11 A diversity of insects that have foraging behaviors in the flowers of *Limnophila aromatica*. A-D: solitary bees insert the head into the flower, collect pollen. (Scale bar = 5 mm.)

Figure 4.12 A diversity of insects that have foraging behaviors in the flowers of *Limnophila geoffrayi*. A-D: solitary bees insert the head into the flower, collect pollen. (Scale bar = 5 mm)

4.5 Seed germination test

Limnophila geoffrayi capsule is ellipsoidal, 1.5 mm width, and 3 mm long (Figure 4.13-A). The seed characters are minute, with black in color and exhibit a variety of shapes, such as oblong, rectangular, and reniform (Figure 4.13-B). According to Ghimire, B., Choi, G. E., Lee, H., Heo, K., and Jeong, M. J. (2017), the seeds of *L. indica* and other species in the same family ranged from ovoid to broad ovoid to sub-spherical. The number of seeds per fruit is approximately 230 seeds. Seed is 0.35 ± 0.04 mm long and 0.23 ± 0.03 mm width (n = 30).

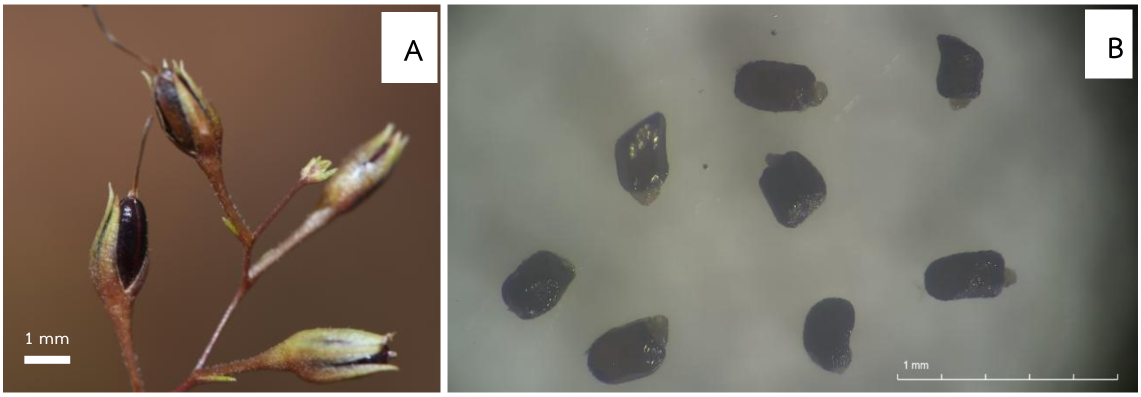

Figure 4.13 Capsule and seed of *Limnophila geoffrayi*. A. capsule, B. variety of seed shape.

The comparison of the seed germination test of *L. geoffrayi* after storage for 0 month and 12 months with various pre-treatments by counting the number of seedlings that germinated daily as determined by the visibility of green (Figure 4.14) then calculated the percentage of seed germination (GP; %), germination index (GI), and mean germination times (MGT; days) are shown in Table 4.3.

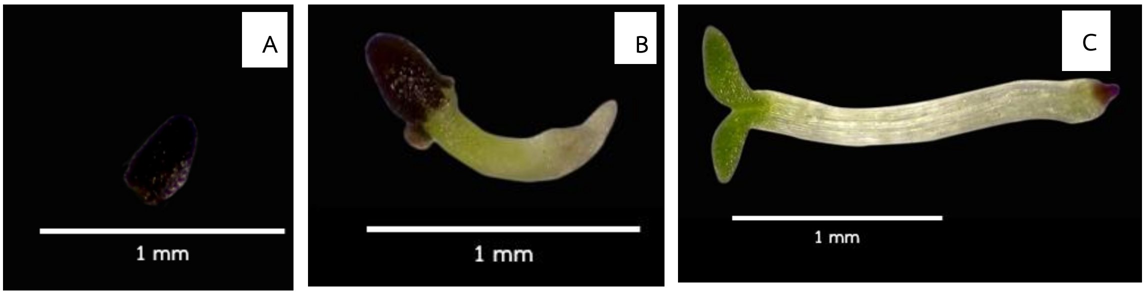

Figure 4.14 The success of seed germination in *Limnophila geoffrayi* assessed by the visibility of green. A. Germination fails, B. Germination success, and C. Seedling.

Table 4.3 One-way ANOVA of the percentage of seed germination (GP; %), germination index (GI), and mean germination times (MGT; days) of *Limnophila geoffrayi* seeds for different treatment series.

Treatment series		GP (%)	GI	MGT (days)
0 month	non-soaking (Control)	35.00±8.55	0.18±0.04	68.30±4.68
	Soaking Water	21.75±4.03	0.12±0.03	66.94±5.68
	250 ppm GA_3	21.50±6.76	0.08±0.03	65.57±6.53
	500 ppm GA_3	19.25±3.50	0.07±0.01	57.39±10.94
	1,000 ppm GA_3	23.50±8.11	0.17±0.06	42.17±16.14
	Sig.	ns	ns	ns
12 months	non-soaking (Control)	42.75±8.84	0.42±0.14	56.29±7.11
	Soaking Water	26.75±5.95	0.31±0.10	54.99±9.16
	250 ppm GA_3	29.75±12.20	0.27±0.09	52.72±13.14
	500 ppm GA_3	15.75±4.23	0.16±0.08	63.79±12.11
	1,000 ppm GA_3	31.75±9.72	0.22±0.09	70.85±6.51
	Sig.	ns	ns	ns

Values are means followed by the standard error (n = 4); ns means not statistically significant (at the α = 0.05 level using DMRT).

The germination percentage (GP) and germination index (GI) of seeds from different storage periods in the five preliminary experimental methods were not significantly different. However, the highest percentages of seed germination and germination index are the seeds stored for 12 months with control (42.75%), the seeds stored for 0 month with control (35%), and the seeds stored for 12 months with 1,000 ppm GA_3 (31.75%). The remaining experiments had germination percentages lower than 30%, which is consistent with other *Limnophila* and other plants in the other family, as Les, D. H. (2018) cited in Panda, M., Satapathy, M. K., and Samal, R. N. (2020), who studied *L. indica* seeds with low germination rates, and the germination study of *Verbascum calycosum* seeds found that the highest germination percentage was only 39-54.5%.

The mean germination times of seeds from different storage periods in the five preliminary experimental methods were also not significantly different. Additionally, the treatment series with the least amount of time to germinate was the seeds stored for 0 month with 1,000 ppm GA_3 (42.17 days), the seeds stored for 12 months with 250 ppm GA_3 (52.72 days), and the seeds stored for 12 months that were soaked with water (54.99 days). The remaining experiments had mean germination times of over 55 days to 70 days (approximately 2 months), which means that *L. geoffrayi* has seed dormancy. Indeed, seeds of *L. geoffrayi* are orthodox due to non-viability loss during storage (Fatima, H., Ishaque, S., Hashim, M., Hano, C., Abbasi, B. H., and Anjum, S., 2023). Similar to other species, such as the study by Hilooglu, M., Sozen, E., Yucel, E., and Kandemir, A. (2018) on *V. calycosum* seeds, which had low germination rates., The study of *Pedicularis olympica* seed, the highest germination rate of this plant occurs after 21 days, possibly demonstrating an interaction of physical and physiological dormancy (Kirmizi, S., Güleryüz, G., Arslan, H., Sakar, F. S., and Kocabıyık, G. A., 2010), and *Scrophularia marilandica* displays physiological dormancy (Nurse, R. E., and Cavers, P. B., 2008).

Gibberellic acid has a crucial role in regulating and enhancing germination in cereal grains and many crop species (Gupta, R. and Chakrabarty, S. K., 2013). However, it did not enhance the germination of *L. geoffrayi* seeds, while enhancing the germination in other plants, such as *V. calycosum* seeds (Hilooglu, M., Sozen, E., Yucel, E., and Kandemir, A., 2018), *P. olympica* seeds (Kirmizi et al., 2010), and *Scrophularia marilandica* seeds (Nurse, R. E., and Cavers, P. B., 2008). I assumed it could be as a result of the unique traits of the *Limnophila* seed coat, which was impenetrable and deteriorated (Ghimire, B., Choi, G. E., Lee, H., Heo, K., and Jeong, M. J., 2017), similar to *P. olympica,* which lacked thick seed coverings, but their endosperm might function as a mechanical limitation to seed germination (Kirmizi, S., Güleryüz, G., Arslan, H., Sakar, F. S., and Kocabıyık, G. A., 2010).

The different treated seeds under seed storage for 0 month, the first seed germination occurred on day 12 in seeds soaked in 500 ppm $GA_{3,}$ day 17 in seeds soaked in 1,000 ppm GA_3, day 24 in seeds soaked in 250 ppm GA_3, day 27 in control, and day 36th in seed soaked in water (Figure 4.15). It was shown that the application of GA_3 stimulated faster germination compared to the control. However, after approximately 60 days of the seed germination test, the control showed faster cumulative germination than the other treatments. It was shown that although GA_3 did not increase the germination percentage as high as the control, it helped to stimulate germination faster.

In addition, stimulating germination by soaking seeds in water did not stimulate the germination of *L. geoffrayi* seeds as well. Normally, seeds soaked in water induces the seeds to germinate faster because water helps increase the activity of enzymes to produce energy and stimulates an increase in the levels of metabolites involved in the germination process (Rodríguez et al., 2015). As studied in other plants by Artola, A., Carrillo-Castañeda, G. and García de los Santos, G. (2003), it was found that water soaking of *Lotus corniculatus* L. seeds could increase the germination rate when the seeds were soaked in water for 19 and 23 hours.

The result of data analysis with two-way analysis of variance (ANOVA) are shown in Table 4.4. Storage period, seed preparation methods (treatment), and their interaction, do not affect the percentage of seed germination (GP) and mean germination times (MGT) values, but storage period has a significant effect on GI values at the $\alpha = 0.05$ level. From the results, it means that storage of *L. geoffrayi* is seeds without doing any further pre-treatment is better way to accelerate germination. I suggest that studying various methods to find the best way to promote germination is required, such as scarification, like the experiment on other plants in the same family, *Veronicastrum sibiricum*. The seeds of this plant treated with different incubation times of cold stratification can enhance the germination (Jang, G. H., Chung, J. M., Rhie, Y. H., and Lee, S. Y., 2022).

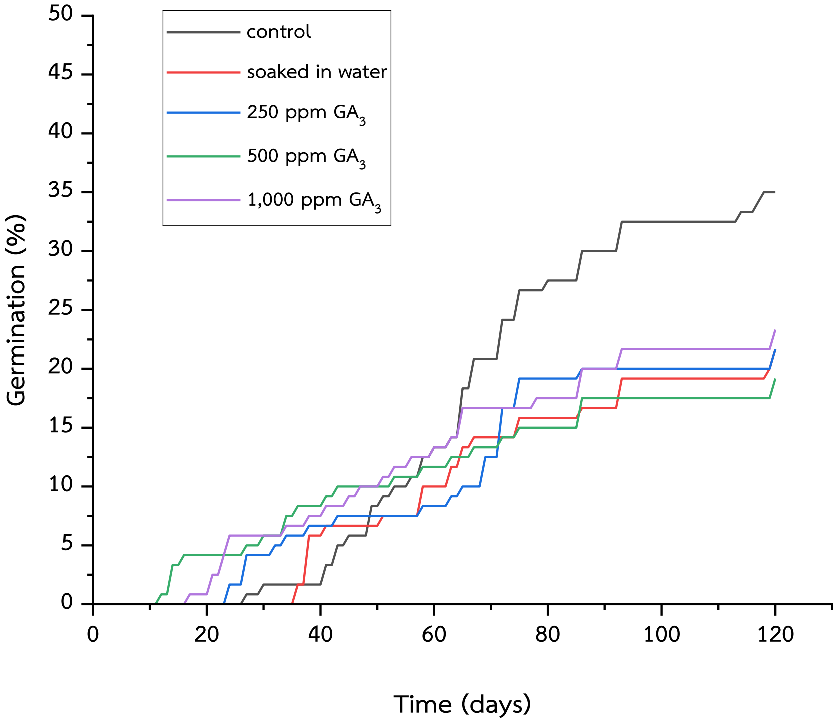

Figure 4.15 Cumulative germination percentage diagrams for *Limnophila geoffrayi* seeds in different treatments under storage seed conditions for 0 month.

Table 4.4 Two-way ANOVA of the percentage of seed germination (GP; %), germination index (GI), and mean germination times (MGT; days) in different storage period, treatment series, and their interaction of *Limnophila geoffrayi* seeds.

Independent variables	Dependent variables	Significant
Treatment series	GP	0.113^{ns}
Different storage period		0.297^{ns}
Treatment series*Different storage period		0.928^{ns}
Treatment series	GI	0.219^{ns}
Different storage period		0.004^{*}
Treatment series*Different storage period		0.695^{ns}
Treatment series	MGT	0.982^{ns}
Different storage period		0.956^{ns}
Treatment series*Different storage period		0.179^{ns}

The ns means not statistically significant, and * means significant at the α = 0.05 level.

In *L. geoffrayi*, different treated seeds were stored for 12 months. The first seed germination occurred on day 9 in seeds soaked in water, in 250 ppm GA$_3$, and in 500 ppm GA$_3$, on day 10 in control, and on day 12 in seeds soaked in 1,000 ppm GA$_3$ (Figure 4.15). It was shown that seeds subjected to 12 months of storage exhibited faster germination than those without a storage period (Figure 4.14). Notably, after approximately 60 days of the seed germination test, the control group showed faster cumulative germination than the other treatments. In this study, it was shown that the storage period increased germination percentage faster than GA$_3$ application, which was consistent with studies in other families, such as Kumar, V., Sharma, S., Sharma, R. K., Kumar, V., and Sharma, S. S. (2024) examined germination test of Scrophulariaceae, *Verbascum thapsus, and* found that germination was significantly affected by storage time, with seeds stored for one year (81%) having a higher germination percentage than 0 year (55%). Similarly, Lu, J. J., Tan, D. Y., Baskin, C. C., and Baskin, J. M. (2017) found that the germination of Brassicaceae, *Isatis violascens,* was significantly affected by storage time, with seeds stored for 6 months having a higher germination percentage than seed with non-stored seeds.

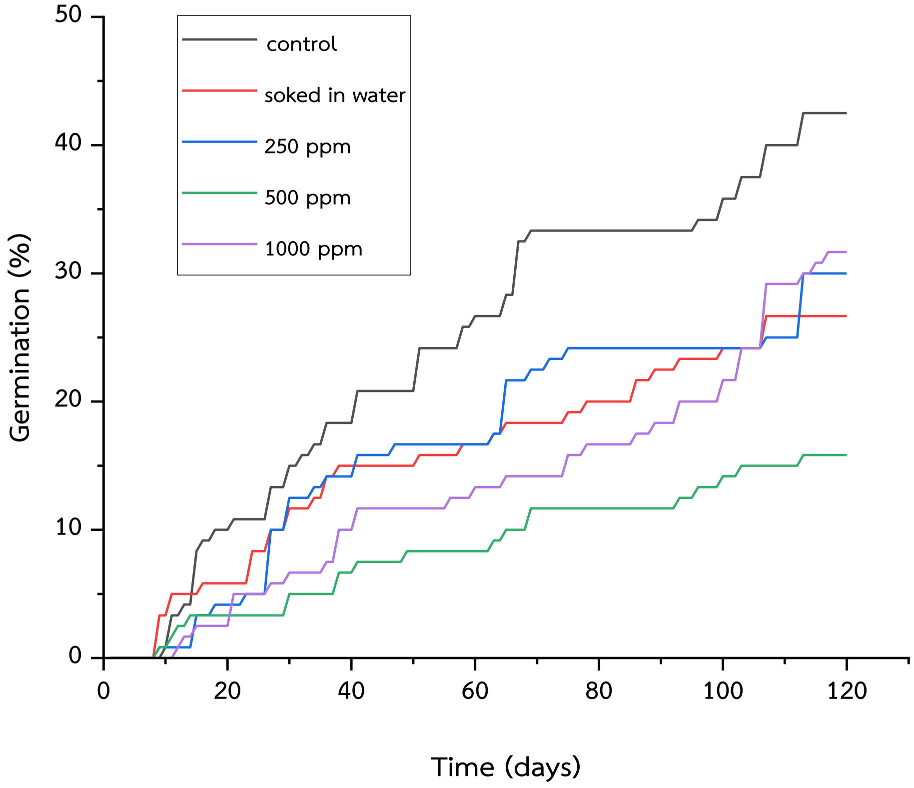

Figure 4.16 Cumulative germination percentage diagrams for *Limnophila geoffrayi* seeds in different treatments under storage conditions for 12 months.

4.6 Effect of BAP on shoot generation of *L. geoffrayi*

The nodal segments of *L. geoffrayi* that were cultured in Murashige and Skoog (MS) medium containing different concentrations of BAP showed 100% regenerated shoots after 8 weeks of culture (Figure 4.17). The first shoot was observed within two weeks in all media. Then after 4 weeks, the number of shoots in control was 1.83±0.38 shoots, the shoot length was 0.53±0.19 cm, with 5.83±0.99 leaves per node. After 6 weeks, all morphological values were increased; the number of shoots in the control was 1.87±0.35 shoots, the shoot length was 0.78±0.22 cm, with 7.10±1.40 leaves per node. The results in other media with different BAP had also increased (Figures 4.18, 4.19, and 4.20).

The addition of BAP into the culture medium significantly increased shoot regeneration in comparison to the control (Table 4.5). However, different BAP concentrations did not result in significant differences in the shoot number. The highest number of shoots was observed at 1 mg/l BAP (52.07±8.22), 0.5 mg/l BAP (46.80±9.94), and 2 mg/l BAP (46.67±9.27), respectively. The study on tissue culture of *Limnophila* is very few. However, Dogan, M. (2019) reported his study on the effective of BAP, to *L. aromatica* shoot generation. BAP concentrations ranging from 0.05, 0.1, and 0.2 mg/l stimulated 21.33, 29.22, and 22.91 shoots per explant, respectively. Indeed, cytokinin (BAP) effectively promotes shoot generation of the genus *Limnophila.*

The longest shoot was found in the media supplemented with 0.5 mg/l BAP (3.38±0.48 cm) and 1 mg/l BAP (3.13±0.29 cm), both of which were significantly longer than the medium added with 2 mg/l BAP (1.81±0.27 cm) and the control (0.95±0.05 cm). It is indicated that BAP not only stimulates to generate the shoot numbers but also shoot length. In *L. aromatica* shoot length were 1.64 cm (0.05 mg/l BAP), 1.92 cm (0.1 mg/l BAP), and 2.11 cm (0.2 mg/l BAP), showing that BAP promoted the shoot length as well (Dogan, M., 2019). Similarly, the number of leaves per shoot showed significant variation among treatments, with the highest values recorded in 1 mg/l BAP (17.53±1.19) and 0.5 mg/l BAP (16.93±2.31), followed by 2 mg/l BAP (11.53±1.18) and the control (8.00±0.26), respectively.

The results indicate that media with 0.5 mg/l BAP is the best concentration that should be used to increase the shoot growth of *L. geoffrayi,* and when BAP concentration was increased, the shoot growth was decreased, consistent with Karatas, M. and Aasim, M. (2015) reported on the study of shoot regeneration of *L. aromatica*. There were reduced shoots per explant when the BAP concentration was raised.

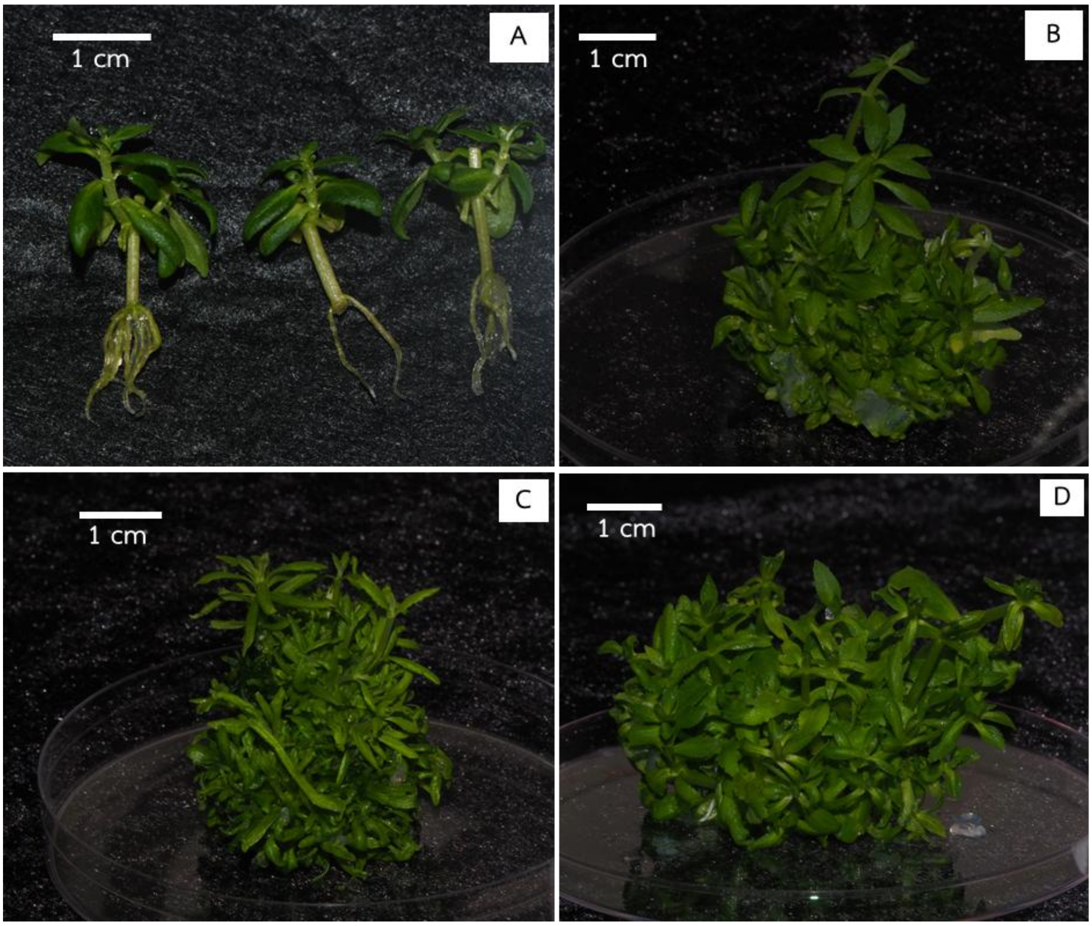

Figure 4.17 Shoot regeneration of *Limnophila geoffrayi* on different concentrations of BAP medium at 8 weeks after culture. A: 0 mg/l BAP, B: 0.5 mg/l BAP, C: 1 mg/l BAP, D: 2 mg/l BAP.

Table 4.5 Effect of different concentrations of BAP on shoots per explant, shoot length, leaves per shoot, roots per explant, and root length of *Limnophila geoffrayi* for 8 weeks (n=15). Values are means followed by the standard error (n = 15); ns means not statistically significant (at the α = 0.05 level using DMRT).

BAP (mg/l)	Shoots/explant	Shoot length (cm)	Leaves/shoot
0	1.87 ± 0.06^{b}	0.95 ± 0.05^{c}	8.00 ± 0.26^{c}
0.5	46.80 ± 9.94^{a}	3.38 ± 0.48^{a}	16.93 ± 2.31^{a}
1	52.07 ± 8.22^{a}	3.13 ± 0.29^{a}	17.53 ± 1.19^{a}
2	46.67 ± 9.27^{a}	1.81 ± 0.27^{b}	11.53 ± 1.18^{b}

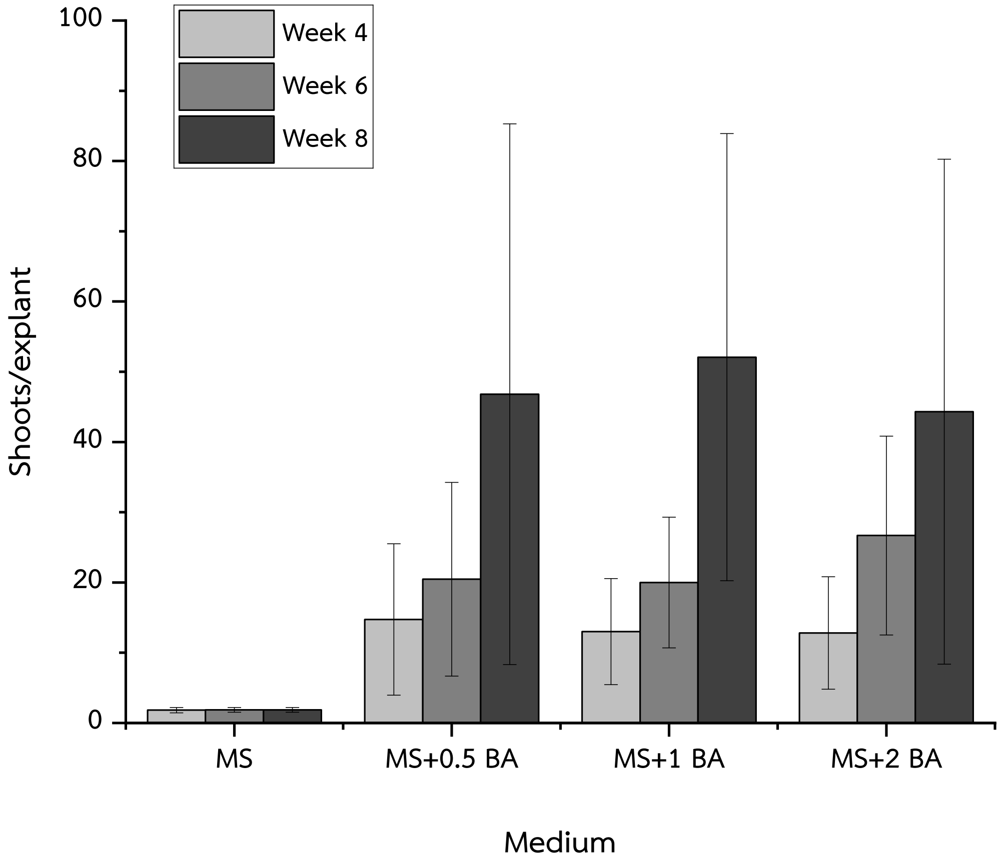

Figure 4.18 The effect of different concentrations of BAP on the number of shoots per explant of *Limnophila geoffrayi* at 4, 6, and 8 weeks.

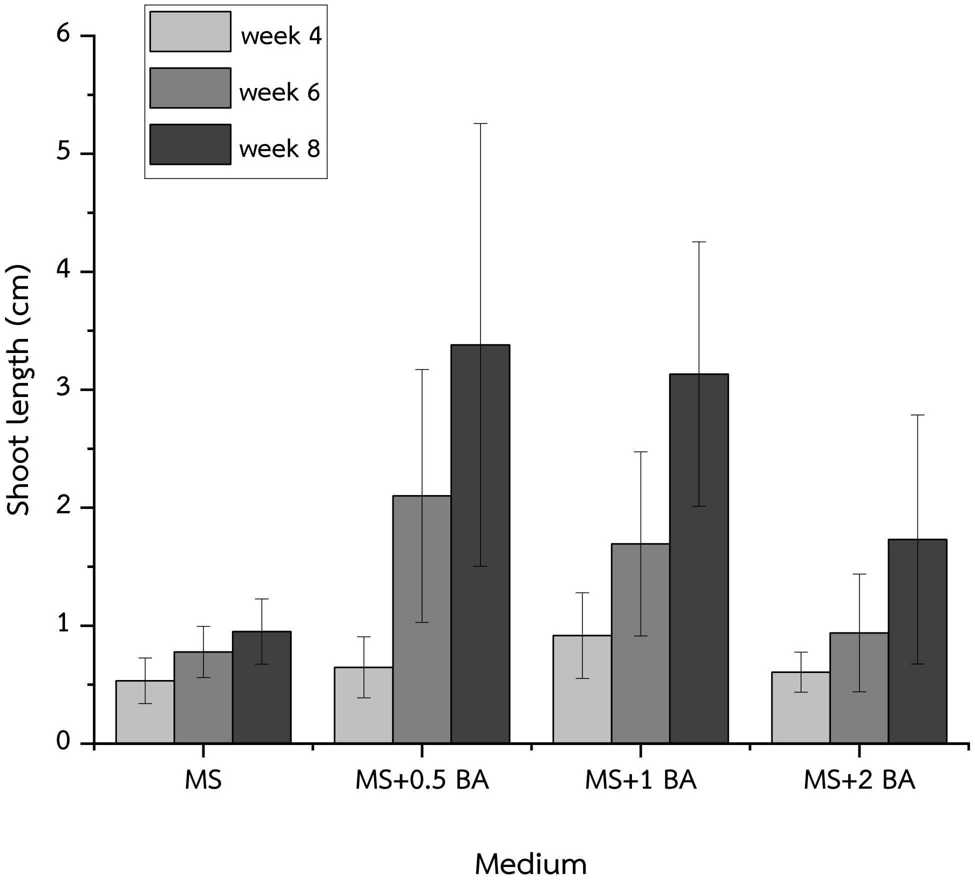

Figure 4.19 The effect of different concentrations of BAP on the shoot length of *Limnophila geoffrayi* at 4, 6, and 8 weeks.

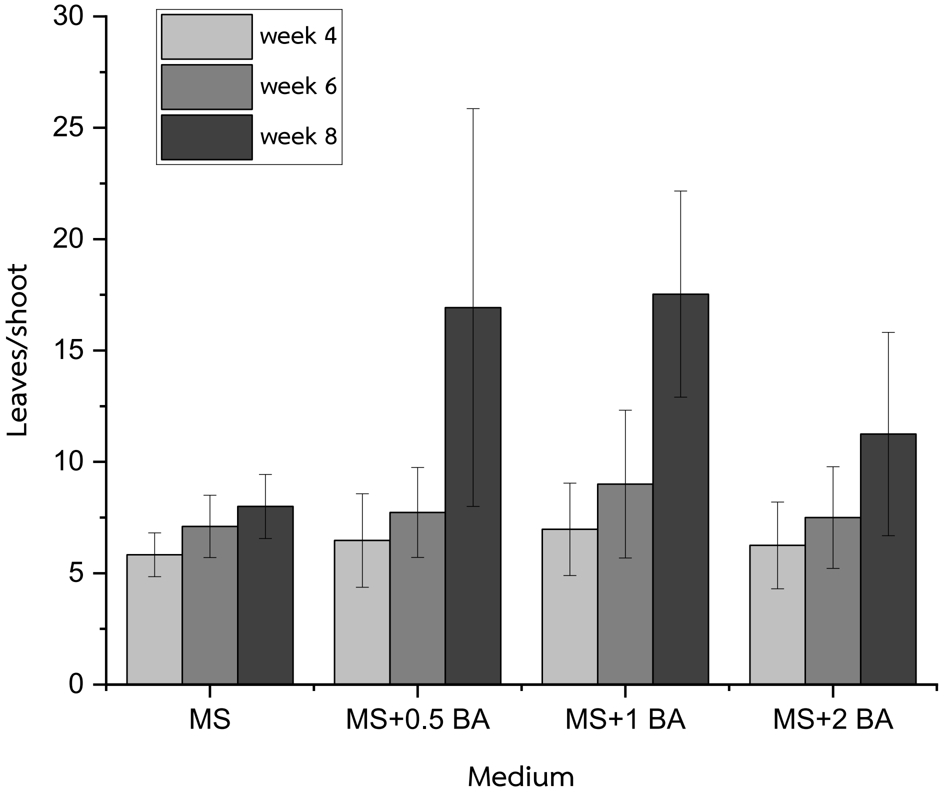

Figure 4.20 The effect of different concentrations of BAP on leaves per explant of *Limnophila geoffrayi* at 4, 6, and 8 weeks.