Analysis of ergot alkaloid gene expression and ergine levels in different parts of *Ipomoea asarifolia*

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Abstract

Ergot alkaloids are renowned for their pharmacological significance and were historically attributed to fungal symbioses with cereal crops and grasses. Recent research uncovered a symbiotic relationship between the fungus *Periglandula ipomoea* and *Ipomoea asarifolia* (Convolvulaceae), revealing a new source for ergot alkaloid synthesis. While past studies have emphasized the storage of both the fungus and alkaloids in leaves and seeds, recent work has found they also occur in other plant parts. This study aimed to ~~(1)~~ examine expression of the *dmaW* gene, which plays a crucial role in ergot alkaloid biosynthesis, and ~~(2)~~ quantify ergot alkaloid levels across various organs and growth stages of *I. asarifolia*. Our findings revealed the highest levels of *dmaW* gene expression in young seeds and young leaves, whereas the highest ergine concentrations were found in mature leaves followed by young leaves. In light of previous studies, we propose three hypotheses to reconcile these conflicting results: ~~(1)~~ the possibility of an inefficient ergot alkaloid biosynthesis pathway, ~~(2)~~ the possibility that different types of ergot alkaloids are produced, and ~~(3)~~ the existence of an ergot alkaloid translocation system within the plant. Furthermore, ergine concentration and ergot alkaloid biosynthesis gene expression were detected in stems, roots, and flowers, indicating that ergot alkaloids are produced and accumulated in all studied parts of *I. asarifolia*, rather than being solely confined to the leaves and seeds, as previously reported.

Keywords

Ergoline alkaloids, morning glory, ~~plant–fungal symbiosis~~, secondary metabolites, vertically transmitted fungi

Introduction

Ergot alkaloids are mycotoxins produced by several species of fungi, particularly those belonging to the Clavicipitaceae family. One well-known representative is the Claviceps purpurea fungus, which infects a wide range of cereal crops and grasses (e.g., rye, wheat, barley, oats, millet, and triticale) and replaces the host grain with dark, ergot-filled structures called sclerotia or ergots (Flieger, Wurst & Shelby, 1997; Krska & Crews, 2008). Interest in ergot alkaloids grew after the discovery of their pharmacological properties and clinical applications (Chen ~~et al~~ *et al*., 2008). For instance, ergometrine is widely used in postpartum treatment as it stimulates uterine contraction (De Costa, 2002). Ergotamine and its derivatives show remarkable anti-migraine efficacy through their interactions with receptors in the central nervous system (Silberstein & McCrory, 2003; Saper & Silberstein, 2006). Additionally, several ergot alkaloids, such as α-dihydroergocryptine, bromocriptine, cabergoline, and pergolide, are effective in treating Parkinson's disease (Bergamasco et al., 2000; Mizuno et al., 2003; Curran & Perry, 2004; Van Camp et al., 2004). Cabergoline is also commonly used as a first-line agent in the treatment of prolactinomas (Corsello et al., 2003; Ono et al., 2008).

In addition to cereal crops and grasses, some plant species in the Convolvulaceae family have been reported to contain toxic bioactive compounds, including ergot alkaloids, indole-diterpene alkaloids, and swainsonine (Lee, Gardner & Cook, 2017; Cook et al., 2019; Beaulieu et al., 2021). Previous research has shown that these ergot alkaloids in Convolvulaceae species are produced by a new genus of symbiotic fungi in the Clavicipitaceae family, *Periglandula* (Kucht et al., 2004; Steiner et al., 2011; Steiner et al., 2012). These fungi contain the dimethylallyl-tryptophan synthase gene (*dmaW*), which is the determinant step in ergot alkaloid biosynthesis (Steiner et al., 2011). The types of ergot alkaloids vary among Convolvulaceous host plant, including chanoclavine, lysergic acid, lysergol, elymoclavine, agroclavine, lysergic acid amide (ergine), lysergic acid α-hydroxyethylamide, ergonovine (ergometrine), penniclavine, setoclavine, festuclavine, ergobalansine, ergonovine, cycloclavine, and others (Beaulieu et al., 2015; Nowak et al., 2016; Steiner & Leistner, 2018). *Ipomoea asarifolia* (Desr.) Roem. & Schult. has been found to contain chanoclavine, lysergic acid amide (ergine), lysergic acid α-hydroxyethylamide, ergonovine (ergometrine), and ergobalansine (Ahimsa-Müller et al., 2007; Steiner & Leistner, 2018), while other host plant species are known to contain different ergot alkaloids.

Periglandula fungi are primarily associated with the leaves and seeds of their host plants (Ahimsa-Müller et al., 2007; Steiner & Leistner, 2018), and ergot alkaloids have been detected in both the leaves and seeds of the plants (Markert et al., 2008; Nowak et al., 2016). Our recent study examined numerous parts of *Ipomoea asarifolia* and discovered *Periglandula ipomoeae* on six out of eight plant parts: young folded leaves, mature leaves, flower buds, mature flowers, young seeds, and mature seeds (Olaranont et al., 2022). However, it is still uncertain which parts of *I. asarifolia* (both fungus-associated and non-associated) contain ergot alkaloids. To confirm the sites of ergot alkaloid biosynthesis in *I. asarifolia*, this study ~~(1)~~ examined gene expression levels of the *dmaW* gene, which is essential for the determining step in ergot alkaloid biosynthesis (Steiner et al., 2006; Ahimsa-Müller et al., 2007), and ~~(2)~~ quantified the amount of ergot alkaloids in different parts of *I. asarifolia*. Such knowledge will better our understanding of symbioses between ergot alkaloid-producing fungi and their host plants.

Materials & Methods

Sample collection

We collected *Ipomoea asarifolia* (Desr.) Roem. & Schult. from its natural habitat in Na Di District, Prachin Buri province, Thailand (14° 08′ N, 101° 44′ E). The plant was identified by P. Traiperm and voucher specimen (Y. Olaranont 07) is stored in the Department of National Parks, Wildlife and Plant Conservation, Bangkok, Thailand. All institutional and national guidelines and legislation were adhered to in the production of this study. Permission to collect plant specimens was granted by private land owners. We collected the following plant parts and developmental stages of interest: young (folded) leaves, mature (opened) leaves, stems, roots, flower buds, mature flowers, young (still green) seeds, and mature (completely ripe) seeds. We kept samples on ice during transport to the lab and, upon arrival, transferred gene expression

94 samples to a -80°C freezer and placed ergot alkaloid quantification samples in an oven at 40°C to
95 dry. Moreover, before conducting gene expression and ergot alkaloid analyses, we first wanted to
96 ensure that the fungus was present on our study plants. We therefore used additional fresh young
97 leaves that we had collected from our study plants to confirm the presence of the fungus via
98 staining (see below) before proceeding with the remaining analyses.
99

100 **Confirmation of fungal presence**

101 We confirmed the presence of the fungus using a staining method with lactophenol cotton blue
102 (Sangeetha & Thangadurai, 2013). We cut fresh young leaves and soaked them in 10% KOH
103 until clear. After a gentle rinse with water, we stained the adaxial surface of the samples with
104 lactophenol cotton blue, which stained the fungal hyphae blue and facilitated observation under a
105 light microscope (Olympus CX21). We previously confirmed that the fungal species was
106 *Periglandula ipomoeae* using molecular analysis (Olaranont et al., 2022). As all examined leaves
107 were found to exhibit the fugus, we proceeded to use our remaining samples for the gene
108 expression and ergot alkaloid analyses.
109

110 **Total RNA extraction and cDNA synthesis**

111 We extracted total RNA from frozen samples (stored at -80°C) of each plant part (five replicates
112 per plant part) using the CTAB method (slightly modified from Morante-Carriel et al. (2014)).
113 We ground each sample with liquid nitrogen and incubated approximately 200 mg of the ground
114 sample with a CTAB extraction buffer (300 mM Tris–HCl, 25 mM EDTA, 2 M NaCl, 2%
115 CTAB, 2% PVPP, and 2% β-mercaptoethanol) at 65°C, before treating samples with
116 chloroform–isoamylalcohol (24:1), 3 M sodium acetate, isopropanol, and 10 M LiCl. We
117 purified total RNA by treating it with DNase using the DNA-free™ DNA Removal Kit
118 (Invitrogen). We then used the purified RNA as a template for synthesizing first-strand cDNA
119 with the iScript™ cDNA Synthesis Kit (Bio-Rad, CA, USA) following manufacturer's protocol.
120 We stored the synthesized cDNA at -20°C until use.
121

122 **Quantitative real-time PCR**

123 We used quantitative RT-PCR (qRT-PCR) to measure expression of the *dmaW* gene compared to
124 three reference genes, all of which are fungal genes. Primers for the *dmaW* gene and reference
125 genes (*actG*, *atp6, tefA*) are listed in Table 1. We developed primers using sequences from the
126 NCBI database. We BLAST searched each primer against the whole genome of *I. asarifolia* to
127 confirm that they are specific to the fungus, with no matches in the plant genome. We conducted
128 qRT-PCR using KAPA SYBR® FAST qPCR Master Mix (2X) with 10 ng of template per
129 reaction (Supplemental file S2). We analyzed each of the five samples per plant part in triplicate
130 using 7,500/7,500 Fast Real-Time PCR Systems (Applied Biosystems, CA, USA). We calculated
131 the relative expression of the *dmaW* gene using the $2^{-\Delta Ct}$ method (Livak & Schmittgen, 2001).
132 The Ct value of the *dmaW* gene was compared to the average Ct value of the three reference
133 genes in each plant part to obtain the ΔCt.

Table 1: ~~List of primers used in this study~~. Some Examples of Genes and Primer sequences

Ergot alkaloid analysis

We quantified ergine concentration (a representative of ergot alkaloids) using high-pressure liquid chromatography (Waters Alliance 2695 HPLC). We dried fresh samples (three replicates per plant part) at 40°C in forced convection oven for 3 days. The dried samples stored in closed container with silica gel before alkaloid extraction. We then ground each sample until it was fine enough to pass through a 425-µm sieve. We soaked approximately 50 mg of the fine powder in 1 ml of gradient-grade methanol (Lichrosolv® by Merck) for 3 days at 4°C and vortexed samples daily (Beaulieu et al., 2015). We filtered the methanol extract of each sample through a 0.45-µm filter and analyzed the extracts via reverse-phase HPLC on a C18 column (Zorbax Eclipse Plus C18: 4.6 × 150 mm i.d., 5 µm; Agilent, USA) with a fluorescence detector set to excitation and emission wavelengths of 310 nm and 410 nm, respectively. We performed the multilinear gradient procedure following Panaccione et al. (2012). We used commercial lysergamide (ergine) as a standard solution (purchased from TRC Canada) and diluted it with acetonitrile to create calibration standards at 50, 500, 1500, 3000, and 6000 ng/ml. We used the Empower Chromatography Data System for quantitative analysis of ergine.

Statistical analysis

We performed all statistical analyses in R version 4.2.1 (R Core Team, 2016). We compared differences in relative gene expression across plant parts using the nonparametric Kruskal-Wallis test with Wilcoxon's multiple comparisons test, given the non-normality of the residuals (Shapiro–Wilk test). We compared differences in ergine concentrations across plant parts using a one-way analysis of variance (ANOVA) with Tukey's multiple comparisons test. Finally, we examined the correlation between relative gene expression and ergot alkaloid concentration using Pearson’s correlation coefficient.

Results

Fungal presence on *I. asarifolia* was confirmed via staining with lactophenol cotton blue. Blue fungal mycelia were detected on the adaxial surface of all young leaves examined (Fig. 1a). Fungal hyphae were observed associating with plant glandular trichomes (Fig. 1b). Our previous experiment confirmed that this fungus is *P. ipomoea* (Olaranont et al., 2022).

Figure 1: Fungal hyphae stained ~~by~~ with lactophenol cotton blue.
(a) Groups of hyphae on the adaxial surface of a young leaf. (b) Close up photo showing the association between fungal hyphae and glandular trichomes (indicated by black arrows) on the leaf surface.

The highest relative expression of the *dmaW* gene was found in young seeds, followed by young leaves, flower buds, mature leaves, mature seeds, mature flowers, stems, and roots, respectively

(Fig. 2). The expression level of the *dmaW* transcript in young seeds was 200 times greater than in roots, which had the lowest expression levels. Comparing young and mature stages revealed higher expression in young leaves, flower buds, and young seeds compared to their mature counterparts (4, 3, and 15 times greater expression, respectively). Our results revealed significant differences in relative expression levels across all plant parts ($\chi2 = 98.154$, $p < 0.01$), except for between mature leaves and flower buds and between mature flowers and mature seeds (Fig. 2). Similar to our *dmaW* results, expression of the three reference genes (*actG*, *atp6*, and *tefA*) was highest in young leaves and young seeds (Fig. S1a). The positive correlation between *dmaW* expression and that of the three reference genes in different plant parts was significant (Fig. S1b; $r = 0.841$, $p = 0.009$).

Figure 2: Relative expression (fold change) of the *dmaW* gene in different plant parts. Expression was relative to the reference genes *actG*, *atp6*, and *tefA*. Plant parts with different letters are significantly different ($p < 0.05$). Abbreviations: YL, young leaf; ML, mature leaf; S, stem; R, root; FB, flower bud; MF, mature flower; YS, young seed; MS, mature seed.

The concentration of ergot alkaloids in *I. asarifolia* was analyzed and quantified through the use of ergine as a representative. Statistical analysis of ergine concentration revealed significant differences across plant parts ($F = 963$, $p < 0.01$). The highest concentration of ergine was found in mature leaves (68.7 µg/g), followed by young leaves, young seeds, mature seeds, stems, flower buds, roots, and mature flowers (48.97, 15.24, 11.71, 4.41, 4.16, 3.20, and 1.72 µg/g, respectively) (Fig. 3). Similar to our gene expression results, young plant parts tended to exhibit higher levels of ergine compared to mature parts, as seen in flowers and seeds. However, mature leaves were found to contain a higher concentration of ergine compared to young leaves. There were no significant differences in concentration among stems, roots, flower buds, and mature flowers, where only minimal amounts of ergine were found (Fig. 3).

Figure 3: Concentration of ergine in different parts of *I. asarifolia*. Plant parts with different letters are significantly different ($p < 0.05$). Abbreviations: YL, young leaf; ML, mature leaf; S, stem; R, root; FB, flower bud; MF, mature flower; YS, young seed; MS, mature seed.

The correlation between relative gene expression and ergine concentration was not significant ($r = 0.266$, $p = 0.525$; Fig. 4). While some plant parts had comparable levels of gene expression and ergine concentrations, other plant parts exhibited unexpected results. Notably, mature leaves exhibited relatively low gene expression but had the highest concentrations of ergine, while young seeds exhibited the highest levels of gene expression but had only moderate concentrations of ergine.

Figure 4: Correlation between relative *dmaW* expression and ergine concentration for the eight plant parts examined in *I. asarifolia*.

214 (r = 0.266, p = 0.525). Abbreviations: YL, young leaf; ML, mature leaf; S, stem; R, root; FB,
215 flower bud; MF, mature flower; YS, young seed; MS, mature seed.
216

217 Discussion

218 This study reveals the relative expression levels of the *dmaW* gene, which is required for the
219 determinant step in ergoline alkaloid biosynthesis, in eight different parts of *I. asarifolia*. The
220 expression of the gene was found in all parts of the plant, with the highest levels in young seeds
221 and young leaves, and the lowest in roots. In addition, ergine concentration was also detected in
222 all studied parts, with the highest concentration in mature and young leaves, and the lowest in
223 mature flowers. This study is the first to develop primers and investigate *dmaW* expression, as
224 well as the first report of ergot alkaloid quantification across multiple parts of the host plant, not
225 just leaves and seeds.
226

227 Differential gene expression of *dmaW* was observed across the studied plant parts of *I. asarifolia*.
228 The three parts of *I. asarifolia* found to have the highest *dmaW* expression levels were young
229 seeds, young leaves, and flower buds. When comparing the expression of the three reference
230 genes to each other, the fold differences across plant organs were similar, which also confirms
231 variation in fungal biomass across different plant parts, with the highest biomass in young seeds
232 and young leaves (Fig. S1a). These results correspond with our previous observation of high
233 densities of fungal hyphae on these same plant parts (Olaranont et al., 2022). Nonetheless, the
234 relative expression of the *dmaW* gene was still found to be exceptionally high in both young
235 seeds and young leaves, even when compared with the average reference gene expression level.
236 These results suggest that these two organs do not just harbor substantial fungal biomass, but that
237 the fungi within these organs also have elevated expression of the ergot alkaloid biosynthesis
238 gene (Fig. S1b). Thus, it appears that ergot alkaloid production is notably amplified in young
239 seeds and young leaves.
240

241 One possible explanation for differential *dmaW* expression is that genes can have specific
242 functions within different plant tissues. Expression level and site are known to vary based on
243 function and environmental conditions (Alberts et al., 2002; Palande et al., 2022). Previous
244 research has demonstrated that environmental or abiotic stress can impact the relative expression
245 of genes involved in secondary metabolite biosynthesis in fungi (Medina et al., 2015). The
246 fungal genus *Metarhizium*, belonging to the Clavicipitaceae family, has been observed to
247 produce ergot alkaloids under certain conditions but not others (Leadmon et al., 2020).
248 Comparing the high level of gene expression in young seeds with the low level in mature seeds
249 observed in our study, it is possible that the soft and moist tissue of young seeds provides a more
250 suitable environment for fungal growth and functioning, compared to the dry conditions
251 experienced in hard mature seeds. Variation in gene expression can also be attributed to the
252 specific functions of the gene. For instance, when *Cucurbit* species were subjected to heat shock,
253 gene expression associated with heat tolerance differed between stems and roots (Ara et al.,

254 2013). The *dmaW* gene is known to play a vital role in synthesizing ergot alkaloids. These
255 alkaloids are believed to serve as a defense mechanism for the plant against insects and
256 herbivores (Jakubczyk, Cheng & O'Connor, 2014; Beaulieu et al., 2021), with the fungi
257 receiving protection and nutrition from the plant in return (Jakubczyk, Cheng & O'Connor, 2014).
258 On the other hand, it is also possible that the fungi produce ergot alkaloids for their own benefit,
259 but these compounds also happen to be beneficial to their host plants. In *I. asarifolia*, a possible
260 explanation for high fungal biomass and *dmaW* gene expression in young seeds and young leaves
261 may be due to the higher nutrient content in these organs compared to others. Further insights
262 will be gained once the specific function of the gene is better understood.
263
264 Our gene expression analysis also revealed two other noteworthy results. First, we detected low
265 levels of *dmaW* expression in both the stems and roots of *I. asarifolia*. This finding is noteworthy
266 given that previous studies were not able to confirm the presence of *Periglandula* in the stems
267 and roots of its host plant through molecular, histochemical, or anatomical techniques (Olaranont
268 et al., 2022). Thus, it appears that stems and roots do host small quantities of the fungus, which
269 can be detected with qRT-PCR due to its higher sensitivity compared to normal PCR (Deprez et
270 al., 2002; Peters et al., 2004). Second, we observed higher expression levels in the young stages
271 of the plant parts, as compared to the mature stages, and this also corresponds with the fungal
272 densities reported in our previous study (Olaranont et al., 2022). However, while the densities of
273 fungal hyphae in flower buds and mature flowers were similar (Olaranont et al., 2022), gene
274 expression levels in flower buds were significantly higher than in mature flowers (Fig. 2). Thus,
275 the higher *dmaW* expression observed in young developmental stages is not solely due to fungal
276 density, and additional research is still needed to understand the drivers of *dmaW* expression.
277
278 We also observed differences in the concentrations of ergine (a representative of ergot alkaloids)
279 found across different parts of the host plant. Previous research has shown that ergot alkaloids
280 are produced by clavicipitaceous fungi, not the host plants themselves (Kucht et al., 2004;
281 Markert et al., 2008), and several Convolvulaceae species were previously found to contain ergot
282 alkaloids, but only the leaves and seeds had been studied (Markert et al., 2008; Beaulieu et al.,
283 2013; Nowak et al., 2016; Beaulieu et al., 2021). Our findings show that leaves are the major
284 accumulation site of ergine, followed by seeds, with other plant parts containing low
285 concentrations of ergine (Fig. 3). Variation in ergot alkaloid concentration across plant parts was
286 expected. A study on *I. asarifolia* and *Turbina corymbosa* (L.) Raf found that even different
287 developmental stages of leaves contain unequal amounts of ergot alkaloids (Steiner et al., 2015).
288 Similarly, Beaulieu et al. (2013) reported that in some Convolvulaceae species the total amount
289 of ergot alkaloids differed significantly in different parts of the seed (cotyledon, embryonic axis,
290 endosperm, and seed coat). The authors suggested that the differential allocation of ergot
291 alkaloids may be influenced by the plant's need for protection against pests and diseases, as these
292 alkaloids are known for their defensive qualities (Beaulieu et al., 2013). If the seedlings are
293 exposed to more pests and diseases above ground, then it is expected that they will allocate more

294 ergot alkaloids to their cotyledons and hypocotyl. Conversely, if the species experiences higher
295 pressure from below-ground pests like nematodes or soil-borne pathogens, then it is anticipated
296 that more ergot alkaloids will be allocated to their roots (Beaulieu et al., 2013). The significantly
297 greater concentrations of ergine found in *I. asarifolia* leaves, compared to stems, roots, flowers,
298 and seeds, therefore suggests that *I. asarifolia* may require the most protection from foliar pests
299 and pathogens.
300
301 Interestingly, we did not observe a correlation between ergot biosynthesis gene expression and
302 ergine concentration across plant parts. Two outliers are particularly noteworthy: young seeds
303 had the highest *dmaW* expression but only moderate ergine concentrations, while mature leaves
304 had low gene expression but the highest concentrations of ergine. Several factors may contribute
305 to these results. First, the biosynthesis pathway of ergot alkaloids is inefficient and it is possible
306 that the necessary precursors needed for ergine production in each part of the plant vary in
307 quantity (Panaccione, 2005). Therefore, even if gene expression is high, as we observed in young
308 seeds, ergine concentrations may be relatively low if there are not enough precursors. Instead,
309 these plant parts may end up containing other intermediates or end products (i.e., other ergot
310 alkaloids) instead of producing high amounts of ergine. Other ergot-producing fungi (e.g.,
311 *Epichloë, Claviceps*, and *Aspergillus*) were also found to have inefficient biosynthesis pathways,
312 which may actually be advantageous if they produce diverse alkaloids within the fungus or its
313 host plant (Beaulieu et al., 2015; Florea, Panaccione & Schardl, 2017).
314
315 Second, our study only examined one type of ergot alkaloid (ergine) due to the inability to obtain
316 the other types of ergot alkaloids. Yet *I. asarifolia* is known to contain other ergot alkaloids as
317 well, such as chanoclavine, lysergic acid alpha-hydroxyethylamide, ergonovine (ergometrine),
318 and ergobalansine (Steiner & Leistner, 2018). Therefore, it is possible that high *dmaW*
319 expression can lead to the biosynthesis of ergot alkaloids other than ergine. The *dmaW* gene
320 initiates the first step of the ergot alkaloid biosynthesis pathway, potentially resulting in a variety
321 of ergot alkaloids beyond just ergine during subsequent steps in the cascade (Liu, Panaccione &
322 Schardl, 2009; Young et al., 2015).
323
324 A third factor that may contribute to the conflicting gene expression and ergine concentration
325 results is differential ergot alkaloid translocation and accumulation within the plant. If the sites
326 of metabolite biosynthesis and accumulation differ, the correlation between gene expression and
327 compound levels can be relatively low (Delli-Ponti, Shivhare & Mutwil, 2021). For example,
328 nicotine alkaloids are synthesized in the root before being transported and accumulated in the
329 aerial parts of the tobacco plant (Katoh et al., 2005). The transportation of ergoline alkaloids
330 between and within *Periglandula* and Convolvulaceous plants is complex and still unclear. The
331 first part of the process involves exchanging sesquiterpene and ergot alkaloids between the fungi
332 and host plant, and the second part involves distributing the ergot alkaloids throughout different
333 parts of the plant (Steiner et al., 2015; Leistner & Steiner, 2018). One study published prior to

the discovery of ergot alkaloid-producing fungi reported that ergot alkaloids in the leaves of *I. tricolor* can be translocated throughout the plant (Mockaitis, Kivilaan & Schulze, 1973). Beaulieu et al. (2013) also reported the differential allocation of ergot alkaloids within Convolvulaceae plant species. Detailed information about how the plant translocates ergot alkaloids is still unknown. Previous studies have reported three families of plant alkaloid transporters, including ATP-binding cassette (ABC) proteins, multidrug and toxic compound extrusion (MATE) transporters, and purine permease (PUP) families (Yazaki, 2006; Shitan, Kato & Shoji, 2014), which could potentially serve as channels for transporting ergot alkaloids in *I. asarifolia*. In addition to these transporters, there are two other potential mechanisms for alkaloid transport: simple diffusion followed by membrane trapping and vesicle-mediated transport (Shitan & Yazaki, 2007; Shitan, Kato & Shoji, 2014). In the case of young and mature *I. asarifolia* leaves, it is also possible that gene expression is high in young leaves, and that ergine continues to accumulate in the leaves as they grow, such that mature leaves contain high concentrations of ergine even though they exhibit low levels of gene expression. The relationship between gene expression and metabolite concentration is complex and further research is required to fully understand ergot alkaloid synthesis, transport, and storage.

Conclusions

It was previously a matter of debate as to where the main site of ergot alkaloid biosynthesis was located. According to previous studies, glandular trichomes on the leaves of the plant were considered to be the prerequisite site for ergoline alkaloids, thus, the leaves were believed to be the biosynthesis site of ergot alkaloids, some of which were then transported to the flowers and seeds of the host plant (Steiner et al., 2015; Leistner & Steiner, 2018). However, our findings indicate that ergot alkaloids are produced and accumulated in most, if not all, parts of *I. asarifolia*. Additionally, there may be a transportation system in place to distribute the compounds throughout the various parts of the plant. Although our study only quantified ergine, a broader analysis using multiple types of ergot alkaloids could offer a clearer understanding of each metabolite's synthesis, transport, and storage. To date, at least 40 species of morning glory have been found to contain ergot alkaloids (Eich, 2008; Beaulieu et al., 2021), and the seeds of these Convolvulaceae species can contain ergot alkaloids at 1000 times greater concentrations than the seeds of grasses infected by other clavicipitaceous fungi (Kucht et al., 2004; Ahimsa-Müller et al., 2007). Considering the beneficial pharmaceutical properties of ergot alkaloids, the biosynthesis gene expression and metabolite concentration across different parts of *I. asarifolia* are particularly noteworthy.

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375

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