INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
20–0271/202x/00–0–000–000
DOI: 10.17957/IJAB/15.1445
http://www.fspublishers.org

Full Length Article

Morphology, Distribution, Density and Chemical Composition of Glandular Trichomes in *Artemisia argyi* (Asteraceae)

Zhanhu Cui[1,3], Xianzhang Huang[2*], Chao Li[2], Zhe Li[4], Mingjie Li[1], Li Gu[1], Li Gao[2], Dahui Liu[5] and Zhongyi Zhang[1*]
[1]*College of Agriculture, Fujian Agriculture and Forestry University, Fuzhou 350002, China*
[2]*Henan Province Key Laboratory of Zhang Zhongjing Formulae and Herbs for Immunoregulation, Nanyang Institute of Technology, Nanyang 473004, China*
[3]*The First People's Hospital of Nanyang Affiliated to Henan University, Nanyang 473010, China*
[4]*Tang-ai Ecological Agriculture Development Limited Liability Company, Nanyang 473400, China*
[5]*Hubei University of Chinese Medicine, Wuhan 430065, China*
[*]For correspondence: hxzgreat@163.com; zyzhang@fafu.edu.cn
Recieved 18 February 2020; Accepted 13 March 2020; Published _______________

Abstract

Artemisiae argyi Levl. et Vant. is a herbaceous plant in the family Asteraceae. For thousands of years, *A. argyi* has been widely used as traditional Chinese medicine. However, the morphological characteristics and secretion of the trichomes on *A. argyi* have not yet been reported. In the present study, the morphology, distribution and density of trichomes at three developmental stages were investigated on the leaves of *A. argyi*, and the chemical constituents of the volatile exudates were identified. Two different morphotypes of glandular trichomes (GTs) and one type of non-glandular trichomes (NGTs) were observed in differential tissues of *A. argyi* for the first time. The results indicated that the density of NGTs on the abaxial surface was higher than on the adaxial surface and that the NGTs were dense at intermediate stages of development. At early development stage, GTs were dense and gradually decreased as the leaves reached maturity in adult leaves. Twelve compounds were extracted and identified from the secretion of GTs in *A. argyi*. A GTs counting method and volatile exudate analysis method were successfully established in this study. Overall, our findings provide a basis for future studies on the effects of environmental stress on the GTs density and inclusion composition of *A. argyi*.

Keywords: *Artemisia argyi*; Trichomes; Morphology; Density; Chemical composition

Introduction

Trichomes are developed from epidermal cells, which consist of single or multiple cells (Schuurink and Tissier 2019). It is a special adaptive structure evolved to cope with biological and abiotic environmental stresses during the long-term evolution of plants. It is widely distributed on the surface of the aboveground parts of terrestrial plants (including gymnosperms, angiosperms and bryophytes) and is one of the important characteristics in taxonomic study. They range in size from a several microns to few centimetres, and can have different shapes (Payne 1978). It can be divided into glandular trichomes (GTs) and non-glandular trichomes (NGTs) according to their structure and function.

GTs are usually multicellular, composed of differentiated basal, stalk and apical cells, which produce a large number of different kinds of metabolites. It is a "cellular chemical factory" for the synthesis of natural products such as terpenoids, flavonoids, polysaccharides and alkaloids (Tissier 2012). Special chemical substances in plant GTs often have unique chemical structures and important biological functions, which play a key role in plant defense. These natural products have diverse structures, complex biosynthesis and extensive biological activities, which are important sources of the discovery of natural drugs. Therefore, more and more attention has been paid to natural products chemistry and other related disciplines.

NGTs are non-secretory protuberance, which are widely found in the epidermis of plant organs on the ground, such as leaves, stems, flowers and fruits. It is a special structure formed by long-term evolution to cope with harsh environment. In complex and changeable environment, NGTs on plant surface plays a more important role than GTs. The morphology and structure of NGTs vary greatly with plant species, so the characteristics of NGTs are regarded as important microscopic identification characteristics in plant medicinal materials. According to current reports, NGTs have the functions of drought resistance, water retention, ultraviolet radiation resistance, insect resistance and pathogen resistance and photosynthesis regulation (Naydenova and Georgiev 2013; Lusa *et al.* 2015; Verma 2017). In some Labiaceae and Verbenaceae plants, NGTs are also involved in the synthesis, storage and release of bioactive substances (Tozin *et al*. 2016; Schuurink and Tissier 2019).

The family Asteraceae is rich in aromatic species used as herbs, folk medicines, fragrances, etc. Many plants in this family have important economic value because they can synthesize secondary metabolites with medicinal value. These medicinal plants, such as *Arnica montana* L., *Arctium lappa* L., *Chrysanthemum lavandulifolium* (Fisch.ex Trautv.) Ling et Shih, Centaurea cyanus L., Tagetes erecta L., and *Achillea wilsoniana* Heimerl ex Hand.-Mazz., are characterized by their leaves and flowers containing flavonoids, saponins, sesquiterpene lactones and coumarins. In addition, GTs exists on the vegetative organs surface of all these plants (Muravnik *et al*. 2019). The genus *Artemisia* L. belongs to the Asteraceae family, which including more than 500 species (Song *et al*. 2019). Although *Artemisia* is the largest genus in the family, the characteristics of trichomes have only been described in a few species. A detailed description of the morphology, anatomy, ultrastructure, and histochemistry, has only been reported for the GTs of *Artemisia annua* (Duke and Paul 1993; Olofsson *et al*. 2012).

Artemisia argyi Levl. et Vant. is called "Aicao" in Chinese and "Gaiyou" in Japanese. It has been widely used in traditional Chinese medicine for thousands of years. *A. argyi* is widely distributed in Asia, Europe and North America (Bora and Sharma 2011). Moxibustion is a kind of thermal therapy, which is still widely used in China, Korea, Japan and other countries (Han *et al*. 2017; Liu *et al*. 2017; Zhu 2018). Moxa floss is a special moxibustion material because of its excellent combustion quality and is made from the dried and processed leaves of *A. argyi* (Zhang *et al*. 2019). *A. argyi* exhibits extensive pharmacological properties and is traditionally used to treat dysmenorrhea, abdominal pain, and inflammation (Chinese Pharmacopoeia Commission 2015). A number of chemical constituents have been isolated and identified from *A. argyi*, including essential oils, flavonoids, terpenes, organic acids, and polysaccharides (Yoshikawa *et al*. 1996; Abad *et al*. 2012; Han *et al*. 2017; Zhang *et al*. 2018). These chemical components have extensive pharmacological properties, which include anti-inflammatory, anti-tumour, antioxidant, anticoagulant, anti-osteoporotic effect, and neuroprotection effects (Seo *et al*. 2003; Zeng *et al*. 2014; Kim *et al*. 2015a–b; Yun *et al*. 2016; Lv *et al*. 2018; Zhang *et al*. 2018). However, studies of the morphological feature and chemical component of GTs for *A. argyi* are not available. Therefore, in this study, we analysed the morphology, distribution, density and secretion of trichomes in the different of vegetative organs of *A. argyi*.

Materials and Methods

Species characteristics

A. argyi is an erect, perennial and herbaceous plant. The taproot is obvious, slightly thick and long, with diameters up to 1.5 cm. The stems are solitary, with a few short branches and obvious longitudinal ribs; they are 80–150 cm long and densely covered with tomentum. The surface of the leaves can be visually confirmed to have short grey and white tomentum, and they also present white glandular spots and small concave points. The back of the leaves is densely covered with grey and white tomentum, and the basal leaves have long stipes. The leaves at the base of the stems are suborbicular or broadly ovate, pinnate and deeply lobed, with petioles that are approximately 0.5–0.8 cm long; the leaves at the middle of the stem are ovate or subrhomboid, 5–8 cm in length, and 4–7 cm wide; the upper leaves are pinnate, semi-lobed, lobed or not divided, but they are elliptic or lanceolate. The capitula are elliptic, with diameters up to 2.5–3.5 mm, and they are sessile or subsessile; the corolla is narrowly tubular, slender in style, and achenes oblong or oblong (Fig. 1). The flowering-fruiting season for *A. argyi* is from July to October.

Plant material

In September to October 2019, samples of aerial parts from *A. argyi* were collected from Nanyang County in Henan Province (33°03′6.56″ N, 112°49′36.91″ E), and identified by Professor Xianzhang Huang from Nanyang Institute of Technology. The voucher specimens of *A. argyi* (NY2019093002) are deposited in the Nanyang Institute of Technology.

Scanning electron microscopy (SEM)

The samples (1×1 cm) from leaves, stems and petioles were fixed in 4% glutaraldehyde solution for 12 h at 4°C. These samples were washed three times with phosphate buffer and post-fixed in 1% osmic acid for 2 h at 4°C. They have been dehydrated at room temperature for 10 min each time with 30, 50, and 70% ethanol respectively, washed two times with isoamyl acetate for 15 min, and critical point–dried with Hitachi CPD-II (Hitachi, Japan). The samples were pasted on the objective table and sprayed with a layer of gold. After that, they were observed with a Hitachi Regulus 8220 (Hitachi, Japan) under different magnification to describe of the morphological feature, density and distribution of GTs and NGTs. To observe the distribution and density of GTs or NGTs on the leaves, stem and petiole of *A. argyi*, GTs on adaxial surface of leaves from three different stages of development (n=30) were analyzed in an area of 1 mm^2 at suitable magnification.

Extraction of the secretion of GTs

The improved method from reported literature (Severson *et al*. 1984; Asai and Fujimoto 2010; Zhou *et al*. 2018) was used to extract volatile exudates from glandular trichomes of *A. argyi*. Fresh leaves of A. argyi (3.5 g) for each group were collected. Three groups of experimental materials were analyzed under the same treatments. Every group samples were dipped 4 times into methylene chloride, and submerged in the solvent for 2 s each time. After that, solvent (containing 20 g anhydrous sodium sulfate) was poured into brown reagent bottle, and stored in the dark for 24 h. The washings were filtered through vacuum filtration and concentrated to 10 mL at 38°C using a rotary evaporator until preparation for analysis.

Gas chromatography/mass spectrometry analysis of the secretion of GTs

The secretion of GTs in *A. argyi* was analyzed using Shimadzu GC-2010 and Shimadzu QP2010 plus MS (Shimadzu Corp., Kyoto, Japan). A capillary column (30 m × 0.25 mm, 0.25 µm film thickness) (Restek, Bellefonte, PA) was used. The volume of sample injecting the machine was 1 μL and the parameter of split ratio was 20:1. The flow rate of helium was 1.3 mL/min. The temperature was increased from 50°C to 90°C at 10°C/min, and continued for about five minutes; increased to 160°C at 10°C/min and continued for about ten minutes; increased to 250°C at 10°C/min and continued for about ten minutes. The temperature of injection was 230°C. The range of scan mass was 40–1000 m/z. The MS was operated in the electron impact mode (70 eV). Compounds were identified by the NIST05 mass spectral library. The relative percentage was determined based on peak area normalization.

Results

Morphology and distribution of trichomes in *A. argyi*

Different morphotypes of trichomes covering the surfaces of the vegetative organs of *A. argyi*. GTs were observed on the leaf (Fig. 2A–C; Fig. 3C–D), stem (Fig. 2D; Fig. 3A) and petiole surfaces (Fig. 2E–F; Fig. 3B). NGTs were also observed on all analysed vegetative organs (Fig. 2A–D).

Based on the morphological survey, the trichomes from *A. argyi* included a total of three types of morphologically distinct trichomes. Among them, there were two types of GTs (I and II) and one type of NGTs. The results showed that types I and II represented two predominant types of GTs (Fig. 4B, C). Only a few type I GTs were observed, as most of them were type II GTs. The morphology of type I GTs was circular, slightly sunken in the middle and made up of four cells (Fig. 3F, H). The length of type I GTs was approximately 40 nm (Fig. 4B). The morphology of type II GTs was non-circular, including two layers, which were made up of eight cells (Fig. 3E, G). Additionally, the bottom was slightly wider than the top. The length of type II GTs was approximately 50 nm (Fig. 4C). Immature GTs were usually distributed in the dent of the leaf epidermis, but mature GTs are often higher than the leaf surface. NGTs are T-shaped and branched (Fig. 4A, D). Trichomes of different stages of development are distributed

Fig. 1: Images of *A. argyi* from aerial parts (A-C).

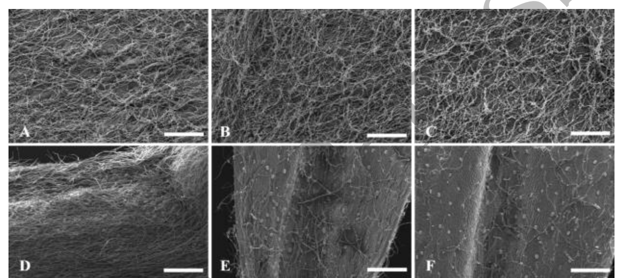

Fig. 2: Distribution of the different types of trichomes on the vegetative organ surfaces of *A. argyi* (SEM). (A-C) Part of the abaxial surface of the young, intermediate and mature leaves. (D) Stem surface. (E-F) Upper and lower petiole surfaces. Bars: 500 µm

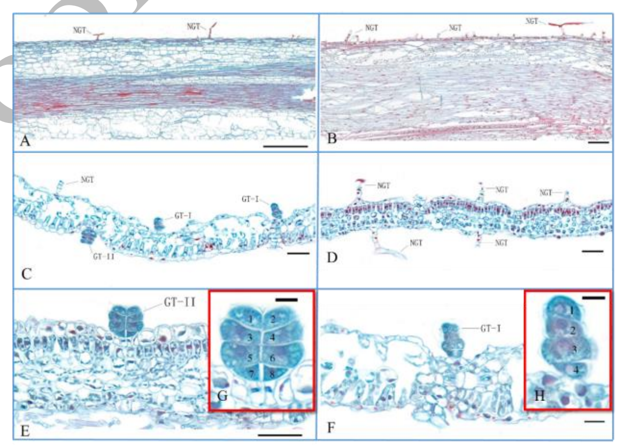

Fig. 3: Light microscopy images of the cross sections of vegetative organs of *A. argyi*: (A) stem; (B) petiole; and (C-D) leaf blades. GT: glandular trichome; NGT: non-glandular trichome. Bars: A, 500 µm; B, 100 µm; C, D, E, 50 µm; F, 20 µm; and G, H, 10 µm

in the abaxial surface of young leaves (Fig. 4A). A type of T-shaped NGTs was observed at the early stage of development (Fig. 4D). Morphological differences between the two types of GTs were obvious during the vigorous growth and senescence periods (Fig. 4B, C, E, F).

Density of glandular trichomes in *A. argyi*

SEM observations revealed that GTs and NGTs were distributed on the abaxial and adaxial surfaces of leaves. However, the density of the trichomes exhibited an obvious

Table 1: Comparative GT density (mm^2) on the adaxial surface of leaves of *A. argyi* at different ontogenetic stages

Fully expanded leaves		
Young *	Intermediate *	Adult *
Adaxial surface	Adaxial surface	Adaxial surface
36.16 ± 6.37	28.23 ± 3.79	17.43 ± 4.24

* Data obtained for n= 30 measurements for each developmental stage. Mean ± SD.

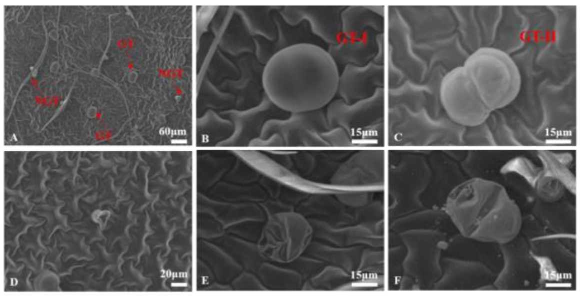

Fig. 4: SEM images of different types of trichomes at different developmental stages in *A. argyi*. A. NGTs at different ontogenetic stages. B-C. Different types of GTs with plump gland surfaces. D. NGTs at the early ontogenetic stage. E-F. Different types of GTs with wrinkled gland surfaces. GT: glandular trichome; NGT: non-glandular trichomes.

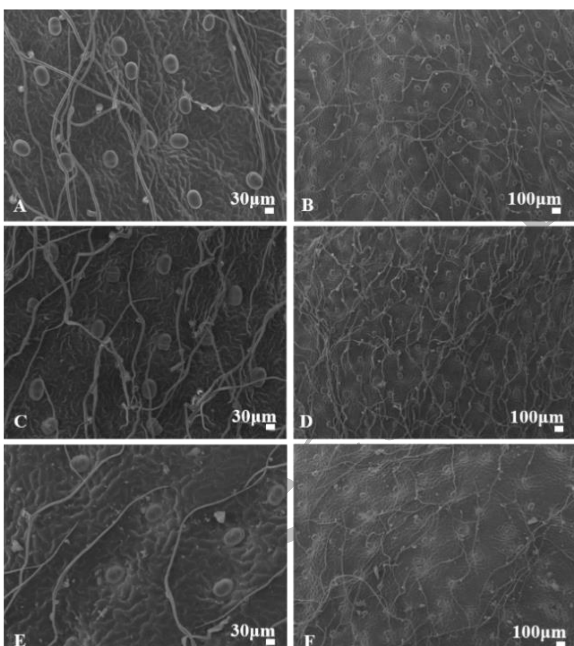

Fig. 5: The adaxial surface of leaves from *A. argyi* at different developmental stages (SEM). A–B: completely unfolded young leaves. C–D: intermediate developmental stage. E–F mature leaves

difference between the abaxial and adaxial surfaces of the leaves. The NGTs density was lower on the adaxial surface than on the abaxial surface, and the NGTs were dense at intermediate stages of development. The density of NGTs on the abaxial surface of the leaves was so high that the GTs could not be counted. The number of trichomes per unit area of mature leaves was less than young leaves. Different morphotypes of trichomes in early ontogenetic stages were not observed in mature leaves. On the adaxial surface, the GTs were dense, and their number gradually decreased as the leaves reached maturity in the early developmental stage (Table 1 and Fig. 5).

Constituents of the secretion of GTs from *A. argyi*

Compositions of the secretion of GTs from *A. argyi* and their relative percentages (%) are shown in Table 2. Twelve compounds were identified from the secretion of GTs from *A. argyi*, including one alkene, one monoterpene, three phenols, four esters, one alcohol, one ketone and one heterocyclic compound. The peak area of these compounds accounted for 41.33% of the total peak area of GC-MS (Fig. 6). (1S,3S,5S)-1-Isopropyl-4-methylenebicyclo[3.1.0]hexan-3-yl acetate and eucalyptol were the major components, and other notable components were 1-naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene)-, [1R-(1.alpha.,4a.beta.,8a.alpha.)]-, 5, 8-dimethyl-1,4,6,7-tetrahydronaphthalene-1,4-dicarboxylic acid, 1,4-dimethyl ester, etc.

Discussion

It is the first report that two different morphotypes of GTs and one type of NGTs that were observed on differential vegetative tissues of *A. argyi* in this study. According to reports, *A. annua* was also covered with peltate GTs and T-shaped NGTs. GT is composed of 10 cells (Duke and Paul 1993; Duke *et al*. 1994; Ferreira and Janick 1995). To date, only a few detailed morphological studies have been carried out on GTs and NGTs structures in the genus *Artemisia* (Kelsey and Shafizadeh 1980; Ascensão and Pais 1982; 1987). However, most plants from the family Asteraceae can produce special aromatic secondary metabolites. In future, more and more in-depth studies on the morphological characteristics and histochemistry of GTs and NGTs for different morphological types of plants in the family Asteraceae will help us to understand the potential role of these structures in these plant species.

The previous studies have shown that large amounts of T-shaped NGTs and few GTs from *A. argyi* exist in moxa floss (Wu *et al*. 2018). The GTs on the surface of leaves and stems could secrete more kinds of volatile oils; therefore, we hypothesized that GTs and NGTs morphology and density are closely associated with the quality of moxa floss. Most of the secondary metabolites in the trichomes of plants are related to key pathway genes, which are specifically or abundantly expressed in GTs specific cells. For example, *A. annua* can synthesize artemisinin, which is largely used as an anti-malarial agent (Graham *et al*. 2010). A few of significant genes, such as *Aldh1*, *CYP71AV1* and *Dbr2*, which are responsible for the biosynthesis of artemisinin, are preferentially expressed in trichomes (Teoh *et al*. 2006; 2009; Zhang *et al*. 2008). To date, no key genes for trichome

Table 2: Components of the secretion of GTs from *A. argyi*

No.	Compounds	Retention time (min)	Relative percentage (%)
1	(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	4.75	0.51
2	Eucalyptol	5.90	8.87
3	Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, (1.alpha.,3.alpha.,5.alpha.)-	9.51	2.46
4	(1S,3S,5S)-1-Isopropyl-4-methylenebicyclo[3.1.0]hexan-3-yl acetate	13.36	10.93
5	Sabinol, 3-methylbut-2-enoate	17.82	0.87
6	1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene)-, [1R-(1.alpha.,4a.beta.,8a.alpha.)]-	20.23	3.72
7	5,8-Dimethyl-1,4,6,7-tetrahydronaphthalene-1,4-dicarboxylic acid, 1,4-dimethyl ester	34.78	3.08
8	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	35.46	1.28
9	2H-Pyran-2-carboxaldehyde, 3,4-dihydro-2,5-dimethyl-	35.82	3.08
10	9,11-Dehydroprogesterone	36.01	2.81
11	2-Butenoic acid, 2-methyl-, dodecahydro-8-hydroxy-8a-methyl-3,5-bis(methylene)-2-oxonaphtho[2,3-b]furan-4-yl ester, [3ar-[3a.al	37.45	2.46
12	Cyclohexanecarboxylic acid, 2-tridecyl ester	39.23	1.26

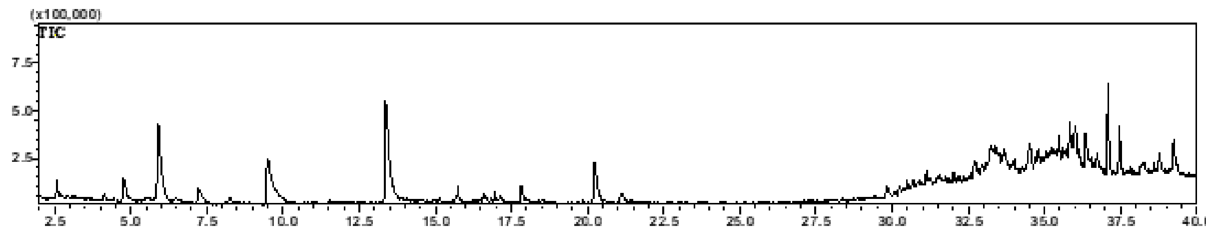

Fig. 6: Total ion chromatogram of GTs volatile exudates from three groups of *A. argyi* by GC-MS

development in *A. argyi* have been reported. In summary, this study has important reference value for further research on the molecular regulation of GT and NGT development for improving the yield and quality of moxa floss, plant classification and agricultural applications in the future.

During the present investigation, the volatile components present in the GTs of *A. argyi* were determined and identified by GC-MS. The secretion of GTs was extracted by methylene chloride. According to a previous report, methylene chloride can quickly extract GTs exudates from plant leaf surfaces (Wagner *et al*. 2004). Moreover, the components cannot penetrate the epidermis and be extracted in internal leaves by using this extraction method. Therefore, compared with other methods, it is an efficient and convenient method for the study of GT secondary metabolism. Twelve compounds were identified from the secretion of GTs of *A. argyi*. Among these compounds, the contents of eucalyptol and (1S,3S,5S)-1-isopropyl-4-methylenebicyclo[3.1.0]hexan-3-yl acetate were higher than the other components in *A. argyi*. To a certain extent, eucalyptol can reflect the quality of medicinal plants and has been used as a quality control marker of *A. argyi* in the Pharmacopoeia of P.R. China (Committee for the Pharmacopoeia of PR China 2015). Previous reports indicated that there were some differences in the contents of total flavonoids, total phenolic acids and bioactive compounds of *A. argyi* in different harvest periods (Xue *et al*. 2019). Only a few compounds of the volatile exudates were isolated from *A. argyi* in this study. This result may be closely associated with the harvest period. In addition, the other chemical components isolated from *A. argyi* exhibit a wide range of biological activities (Song *et al*. 2019), which deserves to be deeply investigated. A number of main components of the volatile exudates were analysed from the whole GTs of *A. argyi* in this experiment. For the next step, differential analysis of the secretory cells in different parts of the GTs of *A. argyi* is expected.

GTs are sites of biosynthesis and storage of large quantities of specialized metabolites (Schuurink and Tissier 2019) and widely exist in plants of the Labiaceae, Compositae, Solanaceae families and plants, such as *A. annua* and *Mentha haplocalyx*. GTs are also called "natural plant factories". GTs can usually adjust their density to adapt to changes in the environment or when under stress (Huchelmann *et al*. 2017). For example, the density of GTs from *Schizonepeta tenuifolia*, *Madia sativa* and *Solanum lycopersicum* exhibited obvious responses to environmental stress, which increased with the aggravation of stress (Gonzáles *et al*. 2008; Galdon-Armero *et al*. 2018; Li *et al*. 2019). A GTs counting method and secretion analysis method were successfully established in this study. Therefore, this study can provide a basis for future studies on the effects of environmental stress on the GT density and inclusion composition of *A. argyi*.

In addition, there are developmental issues beyond the scope of this study that could be used in future research. For example, NGTs are so long and dense on the leaf surfaces of *A. argyi* that the density of NGTs could not be evaluated in this experiment. Establishing an evaluation method for determining the density of NGTs has important practical significance for the quality evaluation of *A. argyi* and moxa floss in the future.

Conclusion

Three different morphotypes of trichomes covering the surfaces of the vegetative organs of *A. argyi*. A number of volatile secondary metabolites were analyzed and identified from the whole GTs of *A. argyi* in this study. The detailed studies on the morphological characteristics and histochemistry of GTs and NGTs from *A. argyi* will contribute us to understand the potential role of trichome structures for the plants in the family Asteraceae.

Acknowledgements

We thank Jiang Chang for assistance in obtaining the SEM micrographs and Yuhang Jiang for GC-MS analysis from College of Horticulture and College of Agriculture, Fujian Agriculture and Forestry University. This study was supported by the National Key Research and Development Program of China (2017YFC1700704), the National Natural Science Foundation of China (81803661) and Science and Technology Open Cooperation Project of Henan Province of China (172106000053).

Author's contribution

XZH, DHL and ZYZ planned the experiments, ZHC, CL, ZL and LG interpreted the results, ZHC and LG made the write up and MJL statistically analyzed the data and made illustrations.

References

Abad MJ, LM Bedoya, L Apaza, P Bermejo (2012). The *Artemisia* L. Genus: A review of bioactive essential oils. *Molecules* 17:2542–2566

Asai T, Y Fujimoto (2010). Cyclic fatty acyl glycosides in the glandular trichome exudate of *Silene gallica*. *Phytochemistry* 71:1410–1417

Ascensão L, MSS Pais (1982). Secretory Trichomes from *Artemisia Crithmifolia*: some ultrastructural aspects. *Bull Soc Bot Fr Actual Bot* 129:83–87

Ascensão L, MSS Pais (1987). Glandular trichomes of *Artemisia campestris* (ssp. Maritima): ontogeny and histochemistry of the secretory product. *Bot Gaz* 148:221–227

Bora KS, A Sharma (2011). The genus *Artemisia*: A comprehensive review. *Pharm Biol* 49:101–109

Chinese Pharmacopoeia Commission (2015). *Pharmacopoeia of the People's Republic of China*. Chinese Medical Science and Technology Press, Beijing, China

Duke MV, RN Paul, HN Elsohly, G Sturtz, SO Duke (1994). Localization of artemisinin and artemisitene in foliar tissues of glanded and glandless biotypes of *Artemisia annua* L. *Intl J Plant Sci* 155:365–372

Duke SO, RN Paul (1993). Development and fine structure of the glandular trichomes of *Artemisia annua* L. *Intl J Plant Sci* 154:107–118

Ferreira JFS, J Janick (1995). Floral morphology of *Artemisia annua* with special reference to trichomes. *Intl J Plant Sci* 156:807–815

Han BS, ZQ Xin, SS Ma, WB Liu, BY Zhang, L Ran, LZ Yi, DB Ren (2017). Comprehensive characterization and identification of antioxidants in Folium *Artemisiae argyi* using high-resolution tandem mass spectrometry. *J Chromatogr B* 1063:84–92

Huchelmann A, M Boutry, C Hachez (2017). Plant glandular trichomes: natural cell factories of high biotechnological interest. *Plant Physiol* 175:6–22

Galdon-Armero J, M Fullana-Pericas, PA Mulet, MA Conesa, C Martin, J Galmes (2018). The ratio of trichomes to stomata is associated with water use efficiency in *Solanum lycopersicum* (tomato). *Plant J* 96:607–619

Gonzáles WL, MA Negritto, LH Suárez, E Gianoli (2008). Induction of glandular and non-glandular trichomes by damage in leaves of *Madia sativa* under contrasting water regimes. *Acta Oecol* 33:128–132

Graham IA, K Besser, S Blumer, CA Branigan, T Czechowski, L Elias, I Guterman, D Harvey, PG Isaac, AM Khan, TR Larson, Y Li, T Pawson, T Penfield, AM Rae, DA Rathbone, S Reid, J Ross, MF Smallwood, V Segura, T Townsend, D Vyas, T Winzer, D Bowles (2010). The genetic map of *Artemisia annua* L. identifies loci affecting yield of the antimalarial drug Artemisinin. *Science* 327:328–331

Kelsey RG, F Shafizadeh (1980). Glandular trichomes and sesquiterpene lactones of *Artemisia nova* (Asteraceae). *Biochem Syst Ecol* 8:371–377

Kim JK, EC Shin, HJ Lim, SJ Choi, CR Kim, SH Suh, CJ Kim, GG Park, CS Park, HK Kim, JH Choi, SW Song, DH Shin (2015a). Characterization of nutritional composition, antioxidative capacity, and sensory at-tributes of Seomae Mugwort, a native Korean variety of *Artemisia argyi* H. Lev. &Vaniot. *J Anal Methods Chem* 2015; Article 916346

Kim JY, MS Lee, JM Baek, J Park, BS Youn, J Oh (2015b). Massive elimination of multinucleated osteoclasts by eupatilin is due to dual inhibition of transcription and cytoskeletal rearrangement. *Bone Rep* 3:83–94

Li K, SJ Li, ZY Zhou, HZ. Yao, Y Zhou, XQ Tang, KC Wang (2019). Effects of drought stress on glandular trichomes, stomatal density and volatile exudates of *Schizonepeta tenuifolia*. *Chin J Chin Mater Med* 44:4573–4580

Liu D, Y Chen, X Wan, NN Shi, LQ Huang, DR Wan (2017). *Artemisiae argyi* folium and its geo-authentic crude drug *qi ai*. *J Tradit Chin Med* 4:20–23

Lusa MG, EC Cardoso, SR Machado, B Appezzato-da-Glória (2015). Trichomes related to an unusual method of water retention and protection of the stem apex in an arid zone perennial species. *AoB Plants* 7: Article plu088

Lv JL, ZZ Li, LB Zhang (2018). Two new flavonoids from *Artemisia argyi* with their anticoagulation activities. *Nat Prod Res* 32:632–639

Muravnik LE, OV Kostina, AA Mosina (2019). Glandular trichomes of the leaves in three Doronicum species (Senecioneae, Asteraceae): morphology, histochemistry, and ultrastructure. *Protoplasma* 256:789–803

Naydenova GK, GI Georgiev (2013). Physiological function of non-glandular trichomes in red clover (*Trifolium pratense* L.). *J Agric Sci Belgrade* 58:217–222

Olofsson LM, A Lundgren, PE Brodelius (2012). Trichome isolation with and without fixation using laser microdissection and pressure catapulting followed by RNA amplification: Expression of genes of terpene metabolism in apical and sub-apical trichome cells of *Artemisia annua* L. *Plant Sci* 183:9–13

Payne W (1978). A glossary of plant hair terminology. *Brittonia* 30:239–255

Schuurink R, A Tissier (2019). Glandular trichomes: Micro-organs with model status? 225: 2251–2266

Seo JM, HM Kang, KH Son, JH Kim, CW Lee, HM Kim, BM Kwon (2003). Antitumor activity of flavones isolated from *Artemisia argyi*. *Planta Med* 69:218–222

Severson RF, RF Arrendale, OT Chortyk, AW Johnson, DM Jackson, GR Gwynn, JF Chaplin, MG Stephenson (1984). Quantitation of the major cuticular components from green leaf of different tobacco types. *J Agric Food Chem* 32:566–570

Song XW, X Wen, JW He, H Zhao, SM Li, MY Wang (2019). Phytochemical components and biological activities of *Artemisia argyi*. *J Funct Foods* 52:648–662

Teoh KH, DR Polichuk, DW Reed, G Nowak, PS Covello (2006). *Artemisia annua* L. (Asteraceae) trichome-specific cDNAs reveal CYP71AV1, a cytochrome P450 with a key role in the biosynthesis of the antimalarial sesquiterpene lactone artemisinin. *FEBS Lett* 580:1411–1416

Teoh KH, DR Polichuk, DW Reed, PS Covello (2009). Molecular cloning of an aldehyde dehydrogenase implicated in artemisinin biosynthesis in *Artemisia annua*. *Botany* 87:635–642

Tissier A (2012). Glandular trichomes: what comes after expressed sequence tags? *Plant J* 70:51–68

Tozin LRDS, SCDM Silva, TM Rodrigues (2016). Non-glandular trichomes in Lamiaceae and Verbenaceaespecies: morphological and histochemical features indicate more than physical protection. *New Zeal J Bot* 54:446–457

Verma SKM (2017). Trichome: role of promoter and cis-regulatory elements, and effect of gamma radiation, UV radiation, methylation, phosphorylation[J]. *Inter J Pure Appl Biol* 5:284–292

Wagner GJ, E Wang, RW Shepherd (2004). New approaches for studying and exploiting an old protuberance, the plant trichome. *Ann Bot* 93:3–11

Wu J, MR Mao, R Xiao, X Chen, MN Zhang, DR Wan (2018). Moxibustion and Moxa floss. *Asia-Pacific Tradit Med* 14:102–104

Xue ZJ, LX Guo, M Guo (2019). Study on difference of chemical constituents of Qiai in different harvest periods. *Chin J Chin Mater Med*. DOI:10.19540/j.cnki.cjcmm.20190830.202

Yoshikawa M, H Shimada, H Matsuda, J Yamahara, N Murakami (1996). Bioactive constituents of Chinese natural medicines. I. New sesquiterpene ketones with vasorelaxant effect from Chinese moxa, the processed leaves of *Artemisia argyi* Levl. et Vant.: Moxartenone and moxartenolide. *Chem Pharm Bull* 44:1656–1662

Yun C, Y Jung, W Chun, B Yang, J Ryu, C Lim, JH Kim, H Kim, SI Cho (2016). Anti-inflammatory effects of artemisia leaf extract in mice with contact dermatitis *in vitro* and *in vivo*. *Med Inflamm* 2; Article 8027537

Zeng KW, S Wang, X Dong, Y Jiang, PF Tu (2014). Sesquiterpene dimer (DSF-52) from *Artemisia argyi* inhibits microglia-mediated neuroinflammation via suppression of NF-kappaB, JNK/p38 MAPKs and Jak2/Stat3 signaling pathways. *Phytomedicine* 21:298–306

Zhang P, B Shi, T Li, Y Xu, X Jin, X Guo, S Yan (2018). Immunomodulatory effect of *Artemisia argyi* polysaccharide on peripheral blood leucocyte of broiler chickens. *J Anim Physiol Anim Nutr* 102: 939–946

Zhang Y, LP Kang, HM Li, XZ Huang, XY Liu, LP Guo, LQ Huang (2019). Characterization of moxa floss combustion by TG/DSC, TG-FTIR and IR. *Bioresource Technol* 288:121516

Zhang Y, KH Teoh, DW Reed, L Maes, A Goossens, DJ Olson, AR Ross, PS Covello (2008). The molecular cloning of artemisinic aldehyde Δ11 (13) reductase and its role in glandular trichome-dependent biosynthesis of artemisinin in *Artemisia annua*. *J Biol Chem* 283:21501–21508

Zhou Y, NY Tang, LJ Huang, YJ Zhao, XQ Tang, KC Wang (2018). Effects of salt stress on plant growth, antioxidant capacity, glandular trichome density, and volatile exudates of *Schizonepeta tenuifolia* Briq. *Intl J Mol Sci* 19; Article 252

Zhu B (2018). On the considerations about heating materials and temperature of moxibustion in clinical practice. *Acupunct Res* 43:63–67