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Variability of *Centella asiatica* (L.) Urb. flower and seed characteristics in Thailand

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Abstract

The conservative components of the plant, including the flower and seed, exhibit minimal response to environmental changes. However, the variability in the flower and seed traits of Asiatic pennywort in Thailand remains poorly understood. This study aimed to categorize the variation in flower and seed characteristics across 15 accessions of Asiatic pennywort. A Completely Randomized Design (CRD) with five replications was employed. In total, 11 qualitative and 9 quantitative traits of the flower and seed were assessed. Multivariate analyses, including Principal Component Analysis (PCA), Hierarchical cluster analysis, and Pearson correlation coefficient analysis, were performed. Variance analysis of the different accessions revealed significant diversity in flower and seed traits. Notably, variability was observed in characteristics such as bract length, calyx length, epigenous disc, seed width, seed length, and the seed length-to-width ratio. Fifteen Asiatic pennywort accessions were grouped into two clusters on the phylogenetic tree, with distance coefficients ranging from 1.00 to 25.00. The first cluster consisted of four accessions, while the second cluster contained eleven accessions, which were further divided into two sub-clusters: sub-cluster 2.1 with a single accession and sub-cluster 2.2 with ten accessions. In terms of seed traits, the second cluster exhibited higher mean values compared to the first cluster. Conversely, the first cluster displayed larger flowers. These findings demonstrate substantial variability in both flower and seed traits among Asiatic pennywort accessions in Thailand. Such variation may serve as a basis for future classification of Asiatic pennywort germplasm.

Keywords: *Centella asiatica*, Characteristic, Flower, Seed, Thailand, Variability

1. Introduction

Asiatic pennywort (*Centella asiatica* (L.) Urb.) is an herbaceous perennial species belonging to the *Apiaceae* family, which is distributed in Asia, Africa, Australia, and America [1]. Asiatic pennywort has been used in Ayurvedic medicine, traditional medicine and folk medicine in India and China for many centuries [2]. In modern medicine, the whole plant of Asiatic pennywort is used as a health tonic for hypertension, the central nervous system (CNS), gastrointestinal diseases, skin diseases, and also for relieving anxiety and improving cognition [3-5]. Gray et al. [6] reported that Asiatic pennywort extracts and their derivatives are used for their antioxidant, anti-inflammatory, cognitive enhancing, and neuroprotective properties. The primary constituents of Asiatic pennywort are pentacyclic triterpene derivatives, including asiaticoside, asiatic acid, madecassoside, and madecassic acid [7-8]. Gray et al. [6] discovered that isoprenoids (sesquiterpenes, plant sterols, pentacyclic triterpenoids, and saponins) and phenylpropanoid derivatives (eugenol derivatives, caffeoylquinic acids, and flavonoids) are among the phytochemicals found in Asiatic pennywort. However, phytochemicals and active ingredient components depended on genetic variability, environmental, and geographical conditions [9-10].

Devkota et al. [11] concluded that the different habitats have a significant effect on the accumulation of active phytochemicals in Asiatic pennywort. According to Puttarak and Panichayupakaranant [12] the cultivated condition and harvesting period affected the pentacyclic triterpene of Asiatic pennywort [9]. In addition, the post-harvest and extraction technology also affected the components of the bioactive compound of Asiatic pennywort, especially asiaticoside. Tripathy and Srivastav [13] revealed that using the vacuum drying process and cold plasma activated water (CPAW) technique could reduce the damaging effects on bioactive compounds in Asiatic pennywort. Furthermore, extraction techniques, solvents, and temperature also affect asiaticoside content [14]. The extraction of bioactive components from Asiatic pennywort leaf deteriorated as the temperature increased [15]. Therefore, different medicinal species, varieties, plant parts, and production systems, including drying methods, extraction techniques, and storage technology, can offer different yields of natural bioactive compounds and help preserve the bioactive compounds of Asiatic pennywort.

Phenotypic variation in plants has been different depending on environmental conditions. The diversity of Asiatic pennywort was identified in many countries, such as India, Madagascar, Sri Lanka, Malaysia, China, Australia, and Thailand [16-24]. Gbolahan et al. [20] reported that 15 accessions of Asiatic pennywort varied in their secondary metabolites, and the variation depended on their genetics and environment. The phenotypic variability of 30 Asiatic pennywort accessions collected in different regions of Thailand was classified into two clusters based on botanic and agronomic characteristics [25]. However, pant et al. [9] discussed that environmental factors, including temperature, carbon dioxide, light concentration, ozone, soil water, soil salinity, and soil fertility, have a significant impact on medicinal plants' physiological, biochemical, and secondary metabolites. Therefore, the Asiatic pennywort classification based on agronomic characteristics might not provide a comprehensive understanding. When including the intraspecific diversity, it was hard to classify by using only botanical and agronomical characteristics. Also, the variability of Asiatic pennywort in Thailand was not classified by flower and seed characteristics, which are the most conservative parts of the plant. Therefore, flower and seed characteristics of Asiatic pennywort might be used to classify and divide closely related populations within and between Asiatic pennywort accessions. Therefore, the objective of this study was to understand the variation of intra-specific national accessions of Asiatic pennywort by using the characteristics of flower and seed morphology.

2. Materials and methods

The 15 accessions of Asiatic pennywort were collected from different locations and habitats in Thailand during October 2019– February 2020. These accessions are derived as those from the northern part (2 accessions), the southern part (1 accession), the northeastern part (7 accessions), and the central part (5 accessions). The plants in each accession were grown under greenhouse conditions at Lamtakong Research Station, Nakhon Ratchasima Province. The experiment was designed using a randomized complete design (CRD) with five replications. The 20 flower and seed characteristics, divided into 9 quantitative characters (bract length, calyx length, anther width, filament length, epigenous disc length, style length, seed width, seed length, and seed length/width ratio) and 11 qualitative characters (bract color, calyx color, calyx pubescence, anther color, filament color, epigenous disc color, epigenous disc pubescence, style color and seed shape, seed color, and seed indumentum), were collected at 3 mounts after transplant. Nineteen of the flower and seed characteristics were evaluated under a stereo microscope (Nikon model SMZ 25), and seed indumentum was evaluated under a scanning electron microscope (JEOL model JCM 6000 Plus). Statistical analysis was done using SPSS program version 26. Significant differences ($p < 0.05$) were analyzed by one-way ANOVA followed by Duncan's multiple range test (DMRT). The correlation coefficient, principal component analysis, and phenotypic distance based on the Euclidean distance coefficient of the 15 accessions of Asiatic pennywort were analyzed with data from the 9 quantitative floral and seed characteristics by using SPSS program version 26 and the Origin 2021 program.

3. Results

3.1 Variability of flower and seeds characteristics of C. asiatica (L.) Urb. in Thailand

The variability of flower and seed characteristics of Asiatic pennywort in Thailand was classified using 9 quantitative and 11 qualitative characters. The result revealed that for the nine quantitative characters, there were highly significant differences ($p<0.01$) in bract length, calyx length, epigenous disc length, seed width, seed length, and seed length/width ratio. The flower characteristics revealed that the longest bract was observed in AP01, AP03, AP06, AP11, AP16, AP21, AP22, AP24, and AP25 accessions, while the longest calyx was observed in AP03, AP07, AP11, AP16, and AP24 accessions. The longest epigenous disc was observed in AP11, AP22, and AP24 accessions (Table 1). The highest seed width was found in AP07, AP11, AP22, AP24, and AP25 accessions, while the longest seed was found in AP02, AP07, AP12, AP21, AP22, AP24, and AP25 accessions. The highest seed length/width ratio was shown in AP02, AP03, AP06, AP12, and AP21 (Table 3).

Table 1 Mean values of flower characteristics of 15 accessions of Asiatic pennywort in Thailand.

AC no.	Bract length (mm)	Calyx length (mm)	Anther width (mm)	Filament length (mm)	Epigenous disc (mm)	Style length (mm)
AP01	1.22[abc]	2.88[def]	0.27	0.44	1.91[bcd]	0.54
AP02	0.99[de]	3.08[cdef]	0.25	0.44	1.57[de]	0.59
AP03	1.38[a]	4.05[a]	0.29	0.49	1.88[cd]	0.63
AP04	1.08[bcde]	3.13[bcdef]	0.27	0.42	1.77[de]	0.55
AP06	1.24[abc]	3.31[bcdef]	0.28	0.39	1.86[cd]	0.70
AP07	1.15[bcde]	3.51[abcde]	0.29	0.50	1.82[cd]	0.67
AP11	1.26[ab]	3.64[abc]	0.31	0.47	2.18[a]	1.07
AP12	1.13[bcde]	3.32[bcdef]	0.29	0.42	1.73[de]	0.51
AP13	1.05[cde]	2.71[f]	0.26	0.40	1.57[e]	0.45
AP16	1.24[abc]	3.80[ab]	0.26	0.46	1.72[de]	0.58
AP21	1.13[bcde]	3.29[bcdef]	0.27	0.46	1.84[cd]	0.61
AP22	0.96[e]	2.66[f]	0.26	0.50	2.03[abc]	0.54
AP24	1.13[bcde]	3.56[abcd]	0.27	0.51	2.14[ab]	0.64
AP25	1.17[bcd]	2.83[ef]	0.26	0.44	1.87[cd]	0.63
AP32	1.24[abc]	3.17[bcdef]	0.27	0.58	1.92[bcd]	0.62
F-test	**	**	ns	ns	**	ns
CV (%)	11.79	15.17	11.82	15.82	10.80	38.81

ns = not significant, ** = highly significant at *p*-value < 0.01, AC no. = accession number

The qualitative characteristics were studied by visual scoring under a stereo microscope, including 11 characteristics (Table 2 and Figure 1). The result revealed that bract color was classified into two groups: 93.3% pinkish-purple bract and 6.7% yellowish-green bract. The result of calyx color revealed that there were 3 groups: 46.7% of greenish-brown calyx, 40.0% of yellowish-green calyx, and 13.3% of greenish-purple calyx. All accessions of Asiatic pennywort were found to have trichromes on the calyx. The anther color included 73.3% of black anthers, 20.0% of brown anthers, and 6.7% of greenish-brown anthers. The filament color revealed three groups, comprising 6.7% brown, 86.7% yellowish-green, and 6.7% purplish green. The epigenous disc color revealed 4 groups, including 73.3% greenish-brown color, 6.7% yellowish-green color, 6.7% purple color, and 13.3% greenish-purple color. All accessions of Asiatic pennywort were found to have trichromes on the epigenous disc. The results for style colors revealed that there were 3 groups, consisting of 13.3% greenish-brown color, 46.7% yellowish-green color, and 40.0% purplish-green color.

Seed shape and seed color were evaluated under a stereo microscope, while seed indumentum was evaluated under a scanning electron microscope (JEOL model JCM 6000 Plus). The results revealed that the seed shape of all accessions was semi-spheroid, and the lateral was compressed. Seed color revealed 5 groups, including 53.3% brown color, 6.7% dark brown color, 20.0% greenish-brown color, 13.3% light brown color, and 6.7% pinkish-brown color. The seed indumentum of Asiatic pennywort revealed that all accessions showed glabrous indumentum. The testa cell pattern of Asiatic pennywort accessions was an irregular pattern with 3–4 longitudinal ridges. The seed coat revealed stomata pores on both sides for all accessions. The arrangement of stomata pores was irregular.

Table 2 Variability of flower characteristics of 15 accessions of Asiatic pennywort in Thailand.

AC no.	Bract color	Calyx color	Calyx trichrome	Anther color	Filament color	Epigenous disc color	Epigenous disc trichrome	Style color
AP01	pinkish purple	greenish brown	present	black	yellowish green	greenish brown	present	green yellowish
AP02	pinkish purple	greenish brown	present	black	purple-green	greenish brown	present	green yellowish
AP03	pinkish purple	yellowish green	present	black	yellowish green	greenish brown	present	brown greenish
AP04	pinkish purple	yellowish green	present	black	yellowish green	purple	present	green yellowish
AP06	pinkish purple	yellowish green	present	black	yellowish green	greenish brown	present	green yellowish
AP07	pinkish purple	yellowish green	present	black	yellowish green	greenish brown	present	purple, green
AP11	yellowish green	yellowish green	present	greenish brown	yellowish green	yellowish green	present	green yellowish
AP12	pinkish purple	purple greenish	present	brown	yellowish green	greenish brown	present	purple, green
AP13	pinkish purple	purple greenish	present	brown	brown	greenish brown	present	brown greenish
AP16	pinkish purple	greenish brown	present	black	yellowish green	greenish brown	present	green yellowish
AP21	pinkish purple	yellowish green	present	brown	yellowish green	greenish purple	present	purple, green
AP22	pinkish purple	greenish brown	present	black	yellowish green	greenish brown	present	purple, green
AP24	pinkish purple	greenish brown	present	black	yellowish green	greenish purple	present	purple, green
AP25	pinkish purple	greenish brown	present	black	yellowish green	greenish brown	present	purple, green
AP32	pinkish purple	greenish brown	present	black	yellowish green	greenish brown	present	green yellowish

AC no. = accession number.

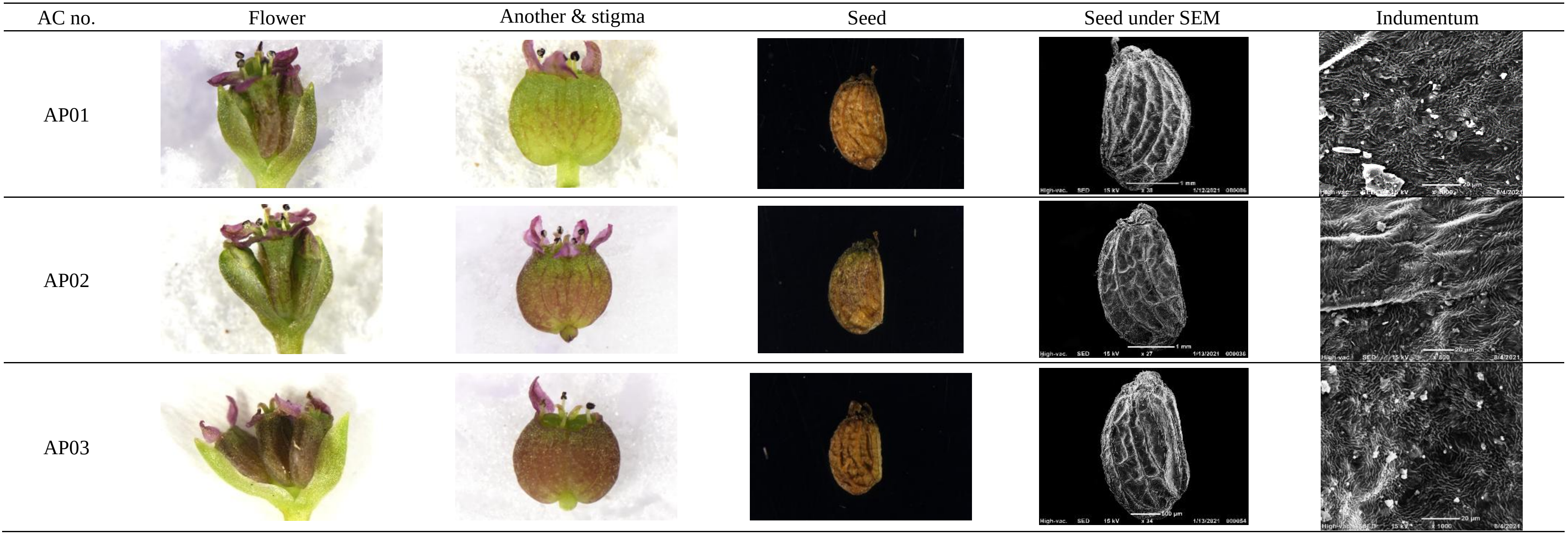

Figure 1 Variability of flower and seed characteristics of 15 accessions of Asiatic pennywort. (AC no. = accession number)

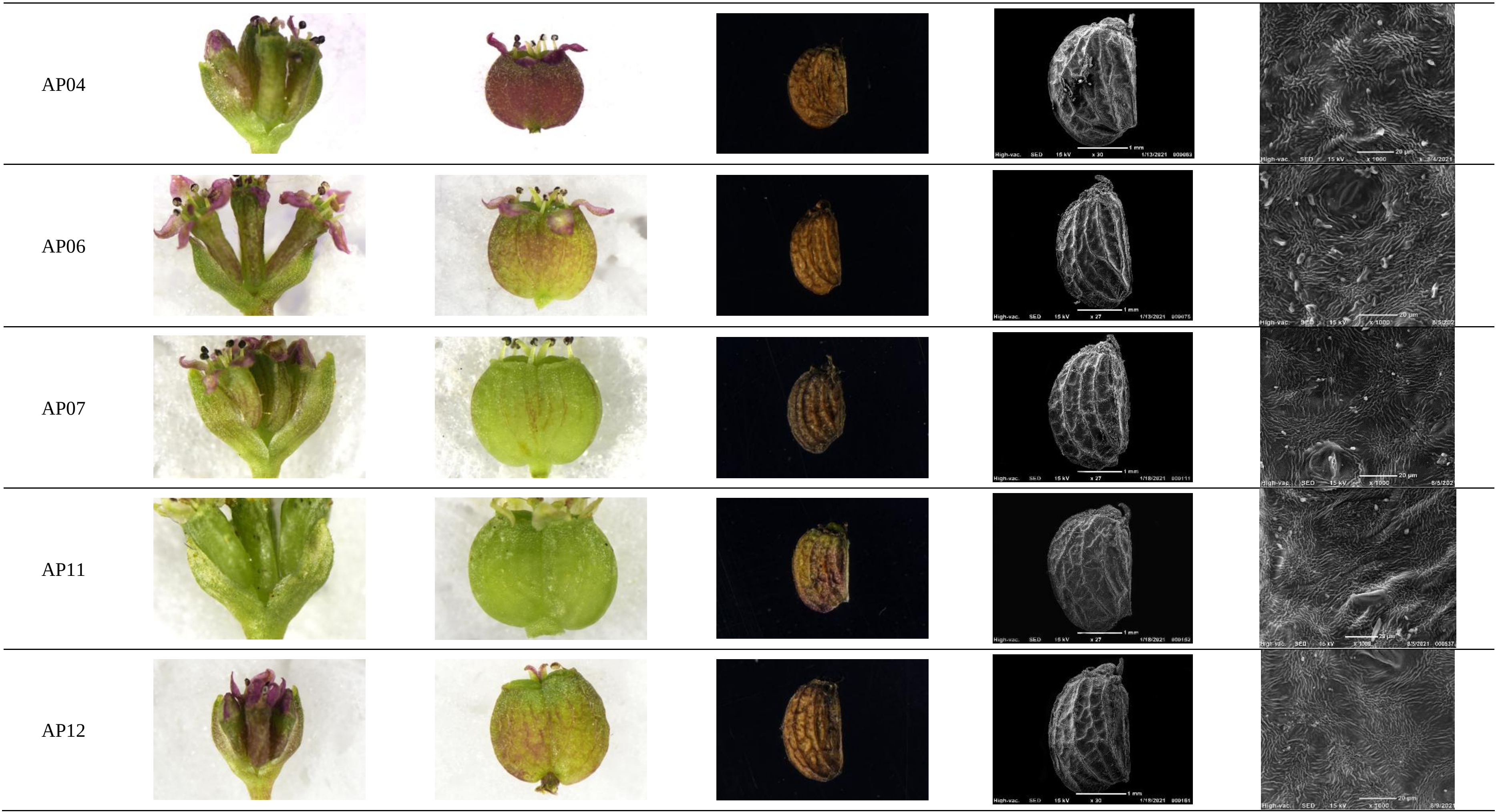

Figure 1 (Cont.) Variability of flower and seed characteristics of 15 accessions of Asiatic pennywort. (AC no. = accession number)

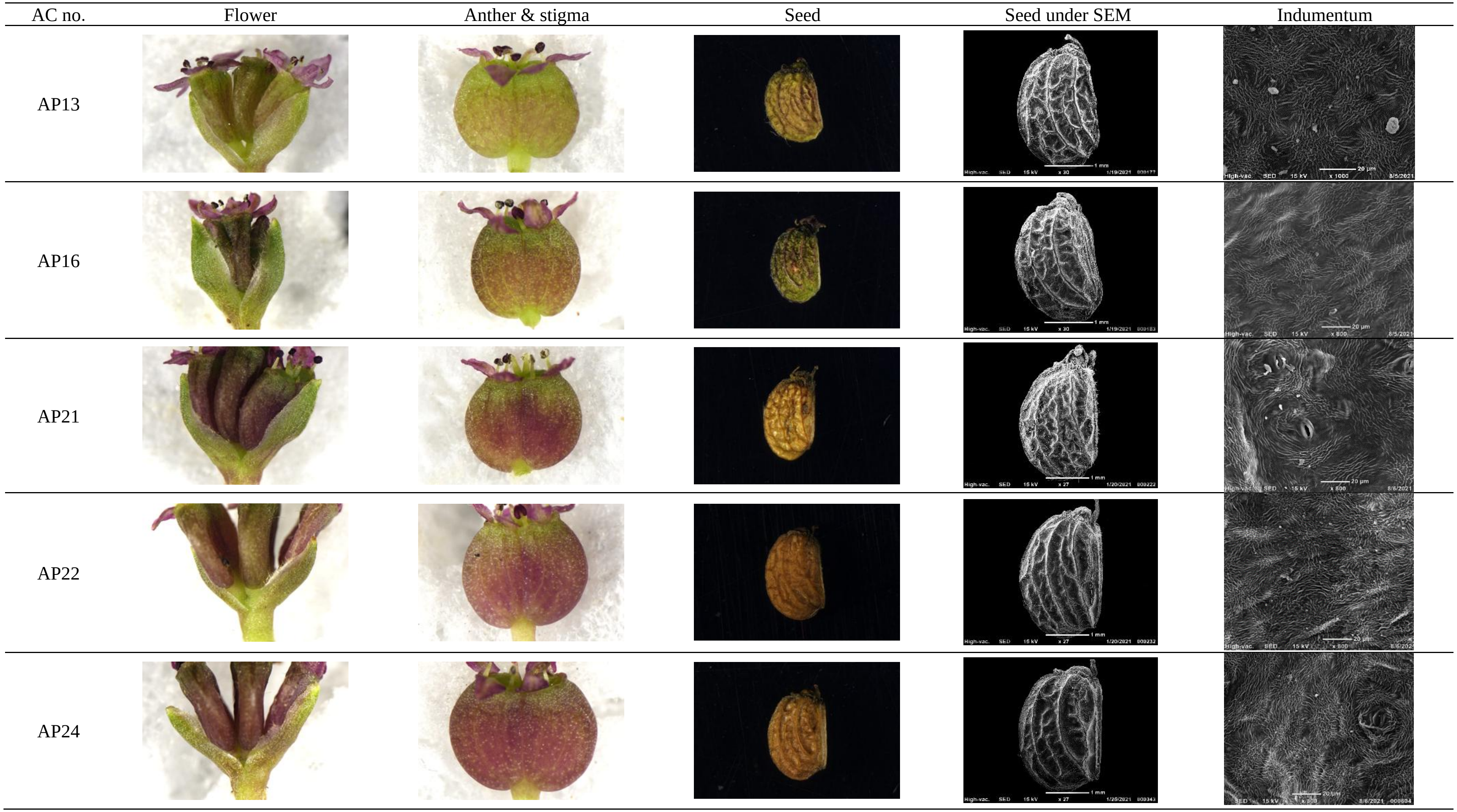

Figure 1 (Cont.) Variability of flower and seed characteristics of 15 accessions of Asiatic pennywort. (AC no. = accession number)

AP25

AP32

Figure 1 (Cont.) Variability of flower and seed characteristics of 15 accessions of Asiatic pennywort. (AC no. = accession number)

Table 3 Mean values and variability of seed characteristics of 15 accessions of Asiatic pennywort in Thailand.

AC no.	Seed width (mm)	Seed length (mm)	Seed length/ width ratio	Seed shape	Seed color	Indumentum
AP01	1.93^{def}	2.90^{bc}	1.50^{bcd}	semi-spheroid	brown	glabrous
AP02	1.92^{def}	3.19^{a}	1.66^{a}	semi-spheroid	brown	glabrous
AP03	1.64^{h}	2.61^{d}	1.59^{abc}	semi-spheroid	brown	glabrous
AP04	1.96^{cde}	2.65^{d}	1.35^{e}	semi-spheroid	brown	glabrous
AP06	1.79^{g}	2.90^{bc}	1.62^{a}	semi-spheroid	brown	glabrous
AP07	2.03^{abcd}	3.03^{ab}	1.49^{cd}	semi-spheroid	dark brown	glabrous
AP11	1.99^{abcde}	2.91^{bc}	1.46^{de}	semi-spheroid	greenish brown	glabrous
AP12	1.97^{bcde}	3.19^{a}	1.62^{a}	semi-spheroid	brown	glabrous
AP13	1.87^{efg}	2.59^{d}	1.39^{de}	semi-spheroid	greenish brown	glabrous
AP16	1.81^{fg}	2.69^{cd}	1.49^{cd}	semi-spheroid	greenish brown	glabrous
AP21	1.87^{efg}	2.99^{ab}	1.61^{ab}	semi-spheroid	light brown	glabrous
AP22	2.08^{abc}	3.09^{ab}	1.49^{cd}	semi-spheroid	brown	glabrous
AP24	2.11^{a}	3.05^{ab}	1.44^{de}	semi-spheroid	brown	glabrous
AP25	2.09^{ab}	3.08^{ab}	1.47^{d}	semi-spheroid	pinkish brown	glabrous
AP32	1.94^{de}	2.88^{bc}	1.48^{d}	semi-spheroid	light brown	glabrous
F-test	**	**	**	-	-	-
CV (%)	7.03	7.63	6.69	-	-	-

** = highly significant at *p*-value < 0.01

3.2 Cluster analysis of flower and seeds characteristics of C. asiatica (L.) Urb. *in Thailand*

The Euclidean distance coefficient was used to cluster 15 accessions of Asiatic pennywort in Thailand using nine flower and seed characteristics (Table 3 and Figure 2). In this dendrogram, the accessions' variability was divided into two clusters with distance coefficients ranging from 1.00 to 25.00. AP03, AP11, AP16, and AP24 were the four accessions that made up the first cluster. The second cluster consisted of 11 accessions, and was classified into two sub-clusters. The 2.1 sub-cluster consisted of 1 accession (AP13), and the 2.2 sub-cluster consisted of 10 accessions (AP01, AP02, AP04, AP06, AP07, AP12, AP21, AP22, AP25, AP32) (Table 4). There was no difference in the means of the nine characteristics between the two clusters, but the seeds and flowers in the first cluster had means that were higher than those in the second cluster. Therefore, the grouping characteristic of these Asiatic pennywort accessions was the size of the flower and seed characteristics (Table 5).

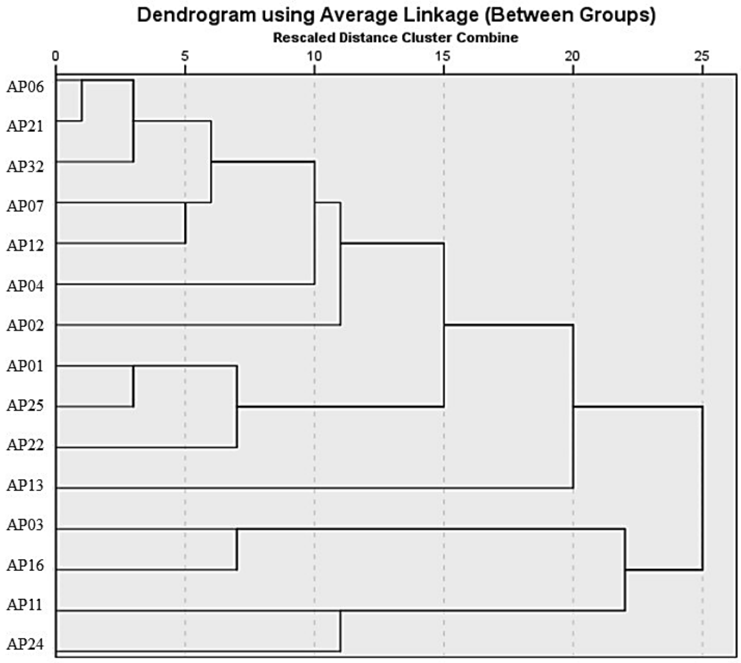

Figure 2 Cluster analysis of 15 Thai Asiatic pennywort accessions based on 9 characteristics of flower and seed**.**

Table 4 Cluster and number of accessions in each cluster of 15 accessions of Asiatic pennywort.

Cluster	Sub-cluster	Number of accession/clusters	Accessions
1	1.1	4	AP03, AP11, AP16, AP24
2	2.1	1	AP13
	2.2	10	AP01, AP02, AP04, AP06, AP07, AP12, AP21, AP22, AP25, AP32

Table 5 Minimum, maximum, mean value and standard deviation (SD) of 15 accessions of Asiatic pennywort.

Traits (mm)	Cluster 1				Cluster 2			
	Min	Max	Mean	Std[1/]	Min	Max	Mean	Std[1/]
seed width	1.64	2.11	1.89	0.21	1.79	2.09	1.95	0.09
seed length	2.61	3.05	2.81	0.20	2.59	3.19	2.95	0.20
seed length/ width ratio	1.39	1.65	1.50	0.07	1.30	1.73	1.52	0.11
bract length	1.13	1.38	1.25	0.10	0.96	1.24	1.12	0.10
calyx length	3.56	4.05	3.76	0.22	2.66	3.51	3.08	0.28
anther width	0.26	0.31	0.28	0.03	0.25	0.29	0.27	0.01
filament length	0.46	0.51	0.48	0.02	0.39	0.58	0.45	0.06
epigenous disc	1.72	2.18	1.98	0.22	1.57	2.03	1.81	0.14
style length	0.58	1.07	0.73	0.23	0.45	0.70	0.58	0.07

[1/] = Standard deviations

3.3 Correlation coefficient and principal component analysis of flower and seeds characteristics of C.asiatica (L.) Urb. *in Thailand*

As for the correlation coefficient of eight Asiatic pennywort flower and seed characteristics in Thailand, the findings revealed a highly significant positive correlation between seed length and width (0.593). Calyx length and bract length had a highly significant positive correlation (0.633). While the relationship between seed width and bract, calyx, and anther length was inverse. Bract length, calyx length, anther width, and filament length all had a negative correlation with seed length. However, Table 6 shows that this character's negative correlation was not very strong. Eight characteristics of flowers and seeds were used in the principal component analysis (Table 7). The outcome showed that two principal components (PCs) had total variability among the studied characters of 52.779% and Eigen values of more than 1.0. The scree plot was used to show how much of the variance was accounted for by each principal component. In comparison to the other 7 PCs, the graph showed that PC1 had the highest level of variability. The investigations of principal components 1 (PC1) and PC2 revealed that seed length and seed width were grouped together in the left upper side of the biplot, suggesting that these parameters had a positive correlation. Meanwhile, other flower characters were grouped together on the right upper side of the biplot, except for bract length. The results of cluster analysis and principal component analysis indicated that the flower and seed characters of Asiatic pennywort were separated into two clusters (Figure 3), in which accessions in cluster 1 have a bigger flower and a smaller seed than accessions in cluster 2 (Figure 2).

Table 6 Correlation between 8 characteristics of 15 accessions of Asiatic pennywort in Thailand.

Traits	Seed width (mm)	Seed length (mm)	Bract length (mm)	Calyx length (mm)	Another width (mm)	Filament length (mm)	Epigenous disc (mm)	Style length (mm)
seed width	1							
seed length	0.593**	1						
bract length	-0.366**	-0.283**	1					
calyx length	-0.294**	-0.228**	0.633**	1				
anther width	-0.021**	-0.045**	0.427**	0.389**	1			
filament length	0.140**	-0.047**	0.035**	0.184**	0.080**	1		
epigenous disc	0.277**	0.046**	0.226**	0.260**	0.248**	0.201**	1	
style length	0.008	0.050**	0.028**	0.094**	0.286**	0.139**	0.236**	1

* Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed).

Table 7 Principal component analysis of 8 characteristics of 15 accessions of Asiatic pennywort in Thailand.

Component	Initial eigenvalues		
	Total	Variance (%)	Cumulative (%)
1	2.440	30.495	30.495
2	1.783	22.284	52.779
3	0.980	12.254	65.033
4	0.933	11.663	76.696
5	0.700	8.747	85.443
6	0.550	6.873	92.316
7	0.340	4.253	96.569
8	0.274	3.431	100.000

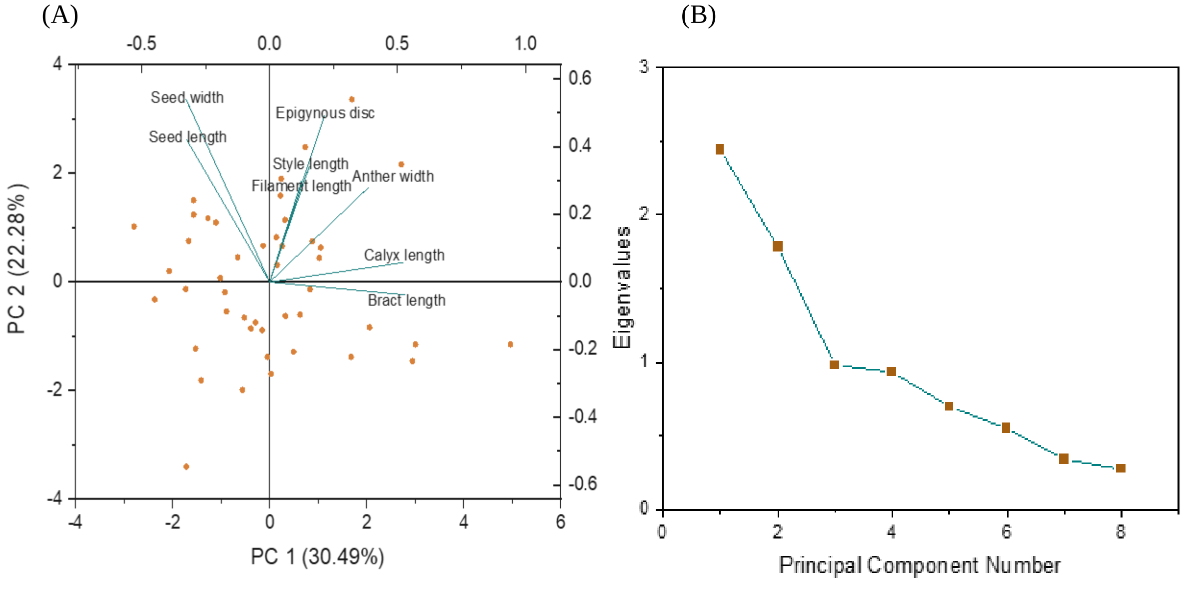

Figure 3 Bi-plot (A) and scree plot (B) of principal component analysis based on flower and seeds variabilities of 15 native Asiatic pennywort in Thailand.

4. Discussions

The diversity of Asiatic pennywort has been classified using phenotypic, chemical component, and genetic variation [19-21], [24-26]. The study of genetic variation used the accuracy method to classify variation in the inter- and intra-population of Asiatic pennywort. However, some methods might be suitable only for preliminary classification. The evaluation of Asiatic pennywort germplasm by using botanic and agronomic characteristics might be suitable. Accordingly, Chachai et al. [25] found that 30 Asiatic pennywort accessions were identified into 2 clusters based on botanic and agronomic characteristics. While in Australia, it was reported that three related species (*C. asiatica* (L.) Urb., *C. cordifolia* (Hook.f.) Nannf., and *C. erecta* (L.f.) Fernald) were classified by using morphological characteristics, molecular markers, and phytochemical composition. The results showed that *C. cordifolia* had the highest triterpene glycosides, phenolics, and antioxidant capacity [24]. Although the morphological characteristics, molecular markers, and phytochemical composition are appropriate to use to identify and classify the germplasm of Asiatic pennywort and related species, however, the expression of morphology and phytochemical components of medicinal plants, including Asiatic pennywort, also depend on both their genetic and growing conditions. Environmental factors such as temperature, carbon dioxide, light concentration, ozone, soil water, soil salinity, and soil fertility affected the physiological, biochemical, and secondary metabolites of medicinal plants [9]. Therefore, the conserved characteristics of the plant, including flower and seed characteristics, may be more beneficial to use for preliminary classification of Asiatic pennywort germplasm. In addition, there is no information about the variability of flower and seed characteristics of Asiatic pennywort. So, this research aimed to identify this variation.

The result of this study shows the variability of flower color and seed color that can be identified in the field. If we used flower color to identify this germplasm, the accessions were classified into two main groups: green and purple flowers. The seed color was variable and divided into six groups, that also related to flower color. However, the seed age of this research was not observed, which might affect the seed color characteristic. According to Novianti et al. [27] the pericarp color of Asiatic pennywort is related to the seed development stage. The differences in seed color were affected by seed germination percentage and time to germinate. So, the color of the seed must be assessed at the same time as the full seed development stage.

The diversity of Asiatic pennywort and growing conditions affect the accumulation of active ingredients. According to the harvesting period [11], James and Dubery [28] reported that the pentacyclic triterpenoids of Asiatic pennywort could be regarded as phytoanticipins due to their antimicrobial activities and protective role against attempted pathogen infections. Madhusudhan et al. [29] revealed that two varieties of Asiatic pennywort (Majjaposhak and Subhodak varieties) in India showed differences in terpenoid content, especially of asiaticoside. Therefore, different medicinal species, varieties, plant parts, and production systems can offer different levels of natural bioactives [9]. This research could serve as preliminary data for identifying Asiatic pennywort germplasm suitable for future breeding programs. Furthermore, further investigation into the selection of accessions with high accumulation of active ingredients is recommended to enhance production for the medicinal industry.

5. Conclusions

The variability is based on the flower and seed characteristics of 15 Asiatic pennywort accessions that were collected from various parts of Thailand. The variability was mainly observed in qualitative and quantitative characteristics such as bract color, calyx color, filament color, epigenous disc color, bract length, calyx length, seed width, and seed length, which are suitable for Asiatic pennywort germplasm characterization. In addition, cluster analysis based on flower and seed characteristics revealed that 15 Asiatic pennywort accessions were classified into two different clusters, with cluster 1 showing the highest mean value in flower characters and cluster 2 showing the highest mean value in seed characters. It’s indicated that accessions in cluster 1 have a bigger flower and smaller seed than accessions in cluster 2. Although flower and seed characteristics of Asiatic pennywort could not be clearly classified and divided closely within and between populations of Asiatic pennywort, however, this study found that Asiatic pennywort revealed variation between accessions based on flower characteristics (bract color, calyx color, filament color, and epigenous disc color). Therefore, it might be concluded that Asiatic pennywort accessions in Thailand have a diversity of flower and seed characters that could be used as a criteria character for classifying Asiatic pennywort germplasm in the future.

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