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Variability of Morphological and Agronomical Characteristics of *Centella asiatica* in Thailand

Namfon Chachai[1], Banthita Pensuriya[1], Techita Pinsuntiae[1], Phattra Pratubkong[1], Janya Mungngam[1], Pongsakorn Nitmee[1], Pongsak Kaewsri[1], Surasit Wongsatchanan[1], Rewat Jindajia[1], Promote Triboun[2] and Jakkrit Sreesaeng[1,*]

[1]*Lamtakong Research Station, Expert Centre of Innovative Agriculture (InnoAg), Thailand Institute of Scientific and Technological Research (TISTR), Pathum Thani 12120, Thailand*
[2]*National Biobank of Thailand (NBT), National Science and Technology Development Agency (NSTDA), Pathum Thani 12120, Thailand*

([*]Corresponding author's e-mail: Jakkritoneku@gmail.com, Jakkrit@tistr.or.th)

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Abstract

The phenotypic variability of 30 accessions of Asiatic pennywort (*Centella asiatica* (L.) Urb.) were collected in different regions in Thailand were evaluated. The growth habit among accessions were identified into 2 types including with 20 accessions of erect from and 10 accessions of flatten from. The leaf arrangement divided into 2 types, including with rosette (20 accessions) and spreading leaf (10 accessions). The variability of morphological and agronomical characteristics experiment was derived by Complete Randomized Design (CRD) with 5 replications. The 11 morphological and agronomical character data were collected at 2 mounts after transplant. The phylogenetic tree manifesting the diversity among 30 accessions based on Euclidean distance coefficient identified by 11 morphological and agronomical characteristics. Analysis of variance revealed high and highly significant difference among accessions for all characteristics, shown a wide range of morphological and agronomical characteristics variability of the Asiatic pennywort germplasm. The phylogenetic tree was classified Asiatic pennywort accessions into 2 different clusters with the distance coefficient ranged from 1.00 - 25.00. The 1st cluster consisted of 8 accessions and the 2nd cluster consisted of 22 accessions. The 2nd cluster was divided into 2 sub- clusters, including with sub-cluster 2.1 consisted of 21 accessions and sub-cluster 2.2 consisted of 1 accession. Mean value of the 2nd cluster was higher than the 1st group all characters except SPAD Index. The result suggested that could be used accession members in the 2nd cluster can be used for future breeding program of Asiatic pennywort in Thailand.

Keywords: Asiatic pennywort, Morphology, Diversity, Correlation, Phenotype

Introduction

Asiatic pennywort was used as a traditional medicine, food and beverage in India, Malaysia, Thailand and others Southeast Asian countries [1-3]. It has been referred to the ancient traditional Chinese Shennong Herbal about 2,000 years ago and used as Ayurvedic medicine in Indian about 3,000 years ago [4]. The information of traditional medicine revealed that Asiatic pennywort is known to be an antioxidant, anti-inflammatory agent, cognitive enhancer and neuroprotective properties [5]. However, Puttarak *et al*. [6] reported that the cognitive function improvement by Asiatic pennywort was not strong evidence to support the cognitive effect, its might be improved alertness and relieve anger. While, Orhan [7] reported that Asiatic pennywort could be suggested to be a desired phytopharmaceutical with neuroprotective effect. According with, Hamidpour *et al*. [8] were found that Asiatic pennywort enhanced memory and cognitive effect.

The research of actives component in Asiatic pennywort were reported in many countries. The actives ingredient of Asiatic pennywort including with saponins, asiaticoside and madecassoside, and their aglycones, asiatic acid and madecassic acids, are the most abundant pentacyclic triterpenoids in all part of Asiatic pennywort [5]. Furthermore, Sudhakaran [9] reported that Asiatic pennywort are richness of several micronutrients, iron, phosphorus, sodium, vitamin C, vitamin A, carotene and dietary fibers.

Although, several nutrients and active ingredients were founded in all Asiatic pennywort accessions. However, the contents of actives ingredient were depended on accessions and the growth conditions. According with Srivastava *et al*. [10] reported that the Asiatic pennywort accessions with differed phytogeographical regions in India revealed different concentration of asiaticoside, madeccasoside and asiatic acid. In addition to the biomass and yield components of Asiatic pennywort were different between accessions. Which, Prasad *et al*. [11] showed that the biomass accumulation of Asiatic pennywort was differed between 4 accessions in India. While, Srithongkul *et al*. [12] reported that genotypic variations and light intensity were affected on growth characteristics and triterpenoid contents, especially on contents of asiaticoside and madecassoside that the main pharmacologically active triterpenoids of Asiatic pennywort.

The diversity of Asiatic pennywort was differed in many regions of tropical and subtropical area. The morphology and actives ingredient components were depended on the genetic variability and geographical conditions. The studies of Asiatic pennywort diversity were reported in different countries such as, China, Malasia, India, Indonesia, Australia and Thailand [11-16]. The Asiatic pennywort germplasm was differed in the level of phenotypic and genetic variability and differed in the level of international and intranational accessions. Alqahtani *et al.* [16] reported that different 3 Centella species (*C. asiatica, C. cordifolia* and *C. Erecta*) were clustered by using methods based on morphological characters, genetic biodiversity, phytochemical compositions and antioxidant activities. According with, Prasad *et al.* [11] reported that the variability of Asiatic pennywort germplasm collected from different part of India shown differed biomass accumulation and madecassoside and asiatic acid content. While, Chandrasekara *et al.* [17] revealed that germplasm evaluation of Asiatic pennywort by using morphological, biochemical and genetic diversity is the essential 1st step in cultivation and breeding efforts. Therefore, the objective of this study aimed to collected and identified the diversity of Asiatic pennywort in Thailand by using morphological and agronomical characteristic data. After that, the collections of Asiatic pennywort could be used to estimate the antioxidants content and used to selected high antioxidant accessions for the future experiments.

Materials and methods

The 30 accessions of Asiatic pennywort were collected from different locations and habitat in Thailand during October, 2019 - February, 2020. These included with the accessions derived from Northern part (4 accessions), Southern part (2 accessions), Northeastern part (17 accessions) and Central part (7 accessions), which divided in 2 habitats were cultivated accession (27 accessions) and natural accession (3 accessions). The plant in each accessions were grown under greenhouse condition at Lamthakong Research Station, Nakhon Ratchasima province. The experiment was designed by used Randomized Complete Design (CRD) with 5 replications. The 11 morphological and agronomical character data (plant height, total leaf number, shoot number, leaf per shoot, leaf length, leaf width, petiole length, stolon number, stolon length, panicle number and SPAD index) were collected at 2 mounts after transplant. SPAD index was measured by chlorophyll meter (SPAD 502, Minolta, Japan) with 3 points of fully expanding leaf (tip, middle and base of leaf). Statistical analysis was done by SPSS program version 19. Significant differences ($p < 0.05$) were analyzed by one-way ANOVA followed by Duncan's multiple range tested (DMRT). The correlation coefficient and phenotypic distance based on Euclidean distance coefficient of 30 accessions of Asiatic pennywort were analyzed with 11 morphology and agronomic characteristics data by SPSS program version 19.

Results and discussion

Locality and habitat of 30 accessions of Asiatic pennywort in Thailand

The diversity of Asiatic pennywort accessions was collected and identified by 11 morphological and agronomical characteristics. The phylogenetic tree manifesting the diversity among 30 accessions of Asiatic pennywort based on Euclidean distance coefficient identified by morphological and agronomical characteristics, analyzed by SPSS program. The location of 30 accessions revealed that Asiatic pennywort were derived from Northern part (4 accessions), Southern part (2 accessions), Northeastern part (17 accessions) and Central part (7 accessions), which divided in 2 habitats were cultivated accession (27 accessions) and natural accession (3 accessions). The habitat of 30 accessions were divided into 2 group as cultivated and natural habitat, while plant growth habit revealed that 2 type including with 20 accessions of erect from and 10 accessions of flatten from. The leaf arrangement of Asiatic pennywort revealed that 2 types, including with rosette (20 accessions) and spreading leaf (10 accessions). The relative of plant growth habit and leaf arrangement was no related. However, the erect growth habit mostly related to the rosette leaf, while flatten growth habit mostly related to the spreading leaf arrangement (**Table 1**).

Table 1 The details of Asiatic pennywort accessions were used in this study.

No.	Code	Location	Habitat	Plant growth habit	Leaf arrangement
1	AP01	Nakhon Pathom Province	Cultivated	Erect	Rosette
2	AP02	Khon Kaen Province	Cultivated	Erect	Rosette
3	AP03	Khunwang District, Chiang Mai Province	Cultivated	Flatten	Spreading
4	AP04	Prachinburi Province	Cultivated	Erect	Rosette
5	AP05	Phang Nga Province	Along the Beach	Flatten	Spreading
6	AP06	Ubon Ratchathani Province	Cultivated	Erect	Rosette
7	AP07	Chiang Rai Province	Cultivated	Erect	Rosette
8	AP08	Nong Bua Lamphu Province	Cultivated	Flatten	Spreading
9	AP09	Chiang Mai Province	Cultivated	Flatten	Spreading
10	AP10	Nakhon Phanom Province	Cultivated	Erect	Rosette
11	AP11	Nakhon Phanom Province	Cultivated	Erect	Rosette
12	AP12	Nakhon Pathom Province	Cultivated	Erect	Rosette
13	AP13	Loei Province	Cultivated	Erect	Rosette
14	AP14	Yasothon Province	Cultivated	Erect	Rosette
15	AP15	Nakhon Ratchasima Province	Cultivated	Erect	Rosette
16	AP16	Nakhon Si Thammarat Province	Cultivated	Erect	Rosette
17	AP17	Nonthaburi Province	Cultivated	Erect	Rosette
18	AP18	Muang District, Nakhon Ratchasima Province	Cultivated	Flatten	Spreading
19	AP21	Phra Nakhon Si Ayutthaya Province	Cultivated	Flatten	Spreading
20	AP22	Bangkok	Cultivated	Erect	Rosette
21	AP23	Yasothon Province	Cultivated	Erect	Spreading
22	AP24	Muang District, Nakhon Ratchasima Province	Cultivated	Flatten	Spreading
23	AP25	Muang District, Nakhon Ratchasima Province	Cultivated	Flatten	Spreading
24	AP28	Chiang Mai Province	Cultivated	Erect	Rosette
25	AP29	Loei Province	Weed in Orchard	Flatten	Spreading
26	AP30	Wang Nam Khiao District, Nakhon Ratchasima Province	Weed in Orchard	Erect	Rosette
27	AP31	Pak Chong District, Nakhon Ratchasima Province	Cultivated	Erect	Rosette
28	AP32	Nong Bua Lamphu Province	Cultivated	Flatten	Rosette
29	AP33	Pak Chong District, Nakhon Ratchasima Province	Cultivated	Erect	Rosette
30	AP34	Nakhon Nayok Province	Cultivated	Erect	Rosette

Department for development of Thai Transitional and Alternative Medicine, Ministry of Public Health was announced Asiatic pennywort as Thailand Champion Herbal Products (TCHP) [2]. However, high quality of raw material that used for drug and herbal product are necessary to classified. While, Sakthipriya *et al*. [18] suggested that a better understanding of genetic diversity and its distribution were essential for its conservation and utilization. Although, the diversity of Asiatic pennywort in Thailand was studied. However, morphology and actives ingredient components were depended on the genetic variability and growing conditions. According with Srithongkul *et al*. [12] reported that 3 different accessions were collected form Nakon Si Thammarat, Rayong and Ubon Ratchathani province, has been differed morphologies and active ingredients. While, Puttarak and Panichayupakaranant [19] revealed that the raw materials were collected from different part of Thailand has differed pentacyclic triterpenes. Also, different part of plants and harvesting time were affected to the pentacyclic triterpenes as well. This indicated that even genetic and growing conditions cause to the differed active ingredients of Asiatic pennywort.

Cluster analysis and variability of morphology and agronomic characteristic of Asiatic pennywort in Thailand

The morphological and agronomical characteristic diversity of Asiatic pennywort accessions were investigated under greenhouse condition. The results revealed that 10 morphological and agronomical characteristics were highly significant at p-value 0.01 and panicle number was significant at p-value 0.05

(**Table 2**). The results of plant height shown the highest value in AP02, AP04, AP06, AP07, AP12, AP13, AP17 and AP22 accessions. Total leaf number revealed that AP02 and AP03 were the highest, about 67 and 41.40 leaf per plant, respectively. While, shoot number per plant was the highest in 12 accessions including with AP02, AP03, AP04, AP08, AP09, AP12, AP13, AP20, AP22, AP23, AP24, AP25 and AP28. However, the highest leaf per shoot shown only 4 accessions including with AP06, AP07, AP18 and AP27. This result revealed that leaf length and leaf width of AP02, AP04, AP06, AP07, AP17, AP20, AP23 and AP24 were large lamina than others. While, the petiole length was shown the longest petiole in AP22. AP02 was the most stolon number, the average about 8.60 stolon/plant. Stolon length revealed that AP02, AP03, AP07, AP08, AP12, AP13, AP17, AP22, AP24 and AP25 were the longest stolon. The panicle number of AP13 was the highest about 38.60 panicles. The result of SPAD index shown the lowest leaf greenness unit in AP13 and AP29.

Table 2 Morphological and agronomical characteristic diversity of 30 accessions of Asiatic pennywort in Thailand.

No.	Code	Plant height (cm.)	Total leaf number	Shoot number	Leaf per shoot	Leaf length (cm.)	Leaf width (cm.)	Petiole length (cm.)	Stolon number	Stolon length (cm.)	Panicle number	SPAD index
1	AP01	7.30^{cdefghi}	12.40^{ghij}	4.40^{efghij}	2.89^{bcd}	3.74^{ef}	2.56^{defgh}	6.26^{defghi}	1.40^{fgh}	20.26^{fghij}	3.60^{b}	16.80^{abc}
2	AP02	10.80^{ab}	67.00^{a}	21.80^{a}	3.16^{bcd}	5.94^{ab}	3.90^{abc}	8.54^{bcd}	8.60^{a}	70.64^{abcd}	11.60^{b}	27.60^{a}
3	AP03	7.43^{cdefghi}	41.40^{ab}	13.20^{abcd}	3.00^{bcd}	3.96^{def}	2.38^{efgh}	5.38^{efghij}	3.40^{bcde}	43.62^{abcdef}	6.80^{b}	17.16^{abc}
4	AP04	10.00^{abc}	39.60^{bcde}	13.20^{abcdefg}	3.06^{bcd}	4.90^{abcde}	3.54^{abcde}	8.50^{bcd}	3.20^{bcdef}	42.20^{cdefgh}	12.80^{b}	24.90^{ab}
5	AP05	5.00^{hijk}	5.75^{ij}	2.75^{ij}	2.60^{bcde}	2.10^{gh}	1.50^{hi}	4.13^{hijk}	0.50^{gh}	7.00^{ij}	0.25^{b}	22.90^{ab}
6	AP06	11.50^{ab}	19.20^{defghij}	6.00^{defghij}	3.81^{ab}	6.46^{a}	4.10^{ab}	10.00^{b}	3.20^{bcdef}	46.86^{cdefg}	8.80^{b}	21.30^{ab}
7	AP07	10.84^{ab}	37.20^{bcdef}	10.40^{bcdefghij}	3.49^{abc}	5.54^{abcd}	3.54^{abcde}	9.30^{bc}	3.60^{bcdef}	70.60^{abcd}	10.00^{b}	18.26^{abc}
8	AP08	6.82^{efghi}	35.80^{bcdefg}	15.60^{abcd}	2.38^{cde}	3.50^{efg}	2.40^{efgh}	5.00^{fghijk}	3.80^{bcdef}	65.40^{abcd}	10.40^{b}	22.66^{ab}
9	AP09	7.00^{defghi}	29.20^{bcdefghi}	12.40^{abcdefgh}	2.50^{bcde}	4.20^{cdef}	2.90^{cdefg}	4.60^{ghijk}	4.40^{bc}	45.00^{cdefg}	8.20^{b}	27.40^{a}
10	AP10	7.00^{defghi}	10.40^{hij}	3.20^{hij}	3.15^{bcd}	4.20^{cdef}	2.40^{efgh}	4.60^{ghijk}	1.40^{fgh}	14.80^{ghij}	3.60^{b}	18.16^{abc}
11	AP11	8.70^{defghi}	23.20^{cdefghij}	8.20^{bcdefghij}	2.87^{bcd}	4.20^{cdef}	2.60^{defgh}	6.10^{defghi}	2.80^{cdefg}	45.50^{cdefg}	9.00^{b}	19.16^{abc}
12	AP12	9.80^{abcd}	34.80^{bcdefgh}	12.60^{abcdefg}	2.76^{bcd}	3.50^{efg}	3.00^{bcdef}	7.20^{cdefg}	4.20^{bcd}	80.60^{a}	10.80^{b}	21.50^{ab}
13	AP13	10.20^{abc}	43.00^{bcd}	17.20^{abc}	2.53^{bcde}	4.20^{cdef}	2.80^{cdefg}	7.40^{bcdef}	5.40^{b}	79.40^{ab}	38.60^{a}	21.60^{ab}
14	AP14	3.56^{jk}	1.43^{j}	1.00^{j}	1.43^{e}	1.21^{h}	1.00^{i}	2.56^{k}	0.00^{h}	0.00^{j}	0.00^{b}	12.58^{bc}
15	AP15	6.40^{fghij}	12.60^{fghij}	5.20^{efghij}	2.39^{cde}	3.10^{fg}	2.10^{fghi}	4.40^{hijk}	1.60^{efgh}	20.20^{fghij}	4.00^{b}	19.60^{abc}
16	AP16	7.75^{cdefgh}	13.75^{fghij}	6.00^{defghij}	1.98^{de}	4.50^{bcdef}	2.63^{defgh}	6.75^{cdefgh}	2.00^{cdefgh}	38.38^{defghi}	8.25^{b}	17.55^{abc}
17	AP17	9.40^{abcde}	29.00^{bcdefghi}	11.20^{bcdefgh}	2.69^{bcde}	5.60^{abc}	3.20^{abcdef}	8.20^{bcde}	2.60^{cdefg}	73.60^{abc}	16.40^{b}	26.34^{a}
18	AP18	3.80^{jk}	16.20^{efghi}	6.20^{defghij}	3.51^{abc}	3.10^{fg}	1.70^{ghi}	3.60^{ijk}	1.40^{fgh}	19.20^{fghij}	3.80^{b}	20.02^{abc}
19	AP21	5.00^{hijk}	18.00^{efghi}	8.00^{cdefghij}	2.57^{bcde}	3.90^{def}	2.20^{fgh}	4.70^{fghijk}	1.80^{defgh}	31.30^{efghij}	9.40^{b}	19.26^{abc}
20	AP22	12.10^{a}	29.40^{bcdefghi}	14.00^{abcde}	2.21^{cde}	6.00^{ab}	3.70^{abcd}	12.80^{a}	3.20^{bcdef}	80.80^{a}	12.80^{b}	18.06^{abc}
21	AP23	5.10^{hijk}	25.00^{bcdefghij}	9.20^{bcdefghij}	3.17^{bcd}	3.50^{efg}	2.10^{fghi}	5.20^{fghijk}	2.20^{cdefgh}	37.50^{defghi}	9.20^{b}	23.48^{ab}
22	AP24	4.60^{ijk}	43.60^{bcd}	16.00^{abc}	2.72^{bcde}	4.20^{cdef}	2.60^{defgh}	5.70^{efghij}	2.40^{cdefgh}	60.80^{abcde}	13.80^{b}	29.04^{a}
23	AP25	7.70^{cdefgh}	40.20^{bcde}	13.80^{abcdef}	3.01^{bcd}	5.10^{abcde}	3.90^{abc}	8.20^{bcde}	3.60^{bcdef}	57.30^{abcde}	12.00^{b}	23.16^{ab}
24	AP28	9.20^{bcdef}	35.40^{bcdefg}	13.60^{abcdef}	2.95^{bcd}	6.00^{ab}	4.30^{a}	8.40^{bcd}	3.20^{bcdef}	51.70^{abcdef}	15.40^{b}	25.00^{ab}
25	AP29	3.00^{k}	13.00^{fghi}	4.25^{efghij}	2.57^{bcde}	2.13^{gh}	1.45^{hi}	3.08^{jk}	1.25^{fgh}	11.13^{hij}	4.25^{b}	15.95^{abc}
26	AP30	4.90^{hijk}	10.20^{hij}	4.00^{fghij}	2.52^{bcde}	4.30^{cdef}	2.80^{cdefg}	6.20^{defghi}	1.40^{fgh}	29.90^{efghij}	6.00^{b}	21.58^{ab}
27	AP31	6.20^{ghij}	23.20^{cdefghij}	5.40^{efghij}	4.57^{a}	3.80^{ef}	2.90^{cdefg}	5.60^{efghij}	1.40^{fgh}	30.20^{efghij}	4.40^{b}	24.92^{ab}
28	AP32	5.30^{hijk}	44.80^{bc}	17.80^{ab}	2.97^{bcd}	4.20^{cdef}	2.50^{efgh}	5.60^{efghij}	2.40^{cdefgh}	42.90^{cdefgh}	4.60^{b}	24.50^{ab}
29	AP33	7.40^{cdefghi}	7.80^{ij}	3.60^{ghij}	2.42^{cde}	3.80^{ef}	2.50^{efgh}	7.30^{cdefg}	0.60^{gh}	11.70^{hij}	2.00^{b}	7.90^{c}
30	AP34	6.14^{ghij}	24.60^{bcdefghij}	9.60^{bcedfghij}	2.89^{bcd}	3.70^{ef}	2.30^{fgh}	4.00^{ijk}	1.60^{efgh}	47.80^{bcdefg}	9.40^{b}	22.48^{ab}
F-test		**	**	**	**	**	**	**	**	**	*	**
% CV		41.1	28.7	30.2	34.0	37.0	38.9	43.5	25.4	21.9	29.4	41.6

* = significant at *p*-value < 0.05, ** = highly significant at *p*-value < 0.01

The result of phenotypic distance based on Euclidean distance coefficient of 30 accessions of Asiatic pennywort were analyzed with 11 morphology and agronomic characteristics data (**Table 3**). The result revealed that, the clustering carried out on the principal components grouped of the 30 Asiatic pennywort accessions into 2 major clusters (**Figure 1**). The majority of accessions in cluster 2 was composed of individuals with a high value for the most characteristics. The cluster 2 was divided into 2 sub-clusters, which composed of sub-cluster 2.1 (21 accessions) and sub-cluster 2.2 (1 accession). The cluster 1 was composed of accessions with a low value for the most characteristics that including with 8 accessions. The clustering pattern indicated that the existence of a significant amount of variability among the Asiatic pennywort collection (**Figure 3**). The cluster one consisted of the AP01, AP05, AP10, AP14, AP15, AP18, AP29 and AP33 accessions that was characterized by the short plants, less leaves, small leaves, low and short stolon. However, the mean of SPAD index was higher that cluster 2. The cluster 2 consisted of the AP02, AP03, AP04, AP06, AP07, AP08, AP09, AP11, AP12, AP13, AP16, AP17, AP21, AP22, AP23, AP24, AP25, AP28, AP30, AP31, AP32 and AP34 accessions that was characterized by the taller plant than cluster 1, large leaves, long petiole, more and long stolon and more panicle. Although, the mean of SPAD index was lower than cluster 1 (**Table 4**).

Table 3 Cluster and number of accessions in each cluster of 30 accessions of Asiatic pennywort.

Cluster	Sub-cluster	Number of accession/cluster	Accessions
1	1.1	8	AP01, AP05, AP10, AP14, AP15, AP18, AP29, AP33
2	2.1	21	AP03, AP04, AP06, AP07, AP08, AP09, AP11, AP12, AP13, AP16, AP17, AP21, AP22, AP23, AP24, AP25, AP28, AP30, AP31, AP32, AP34
	2.2	1	AP02

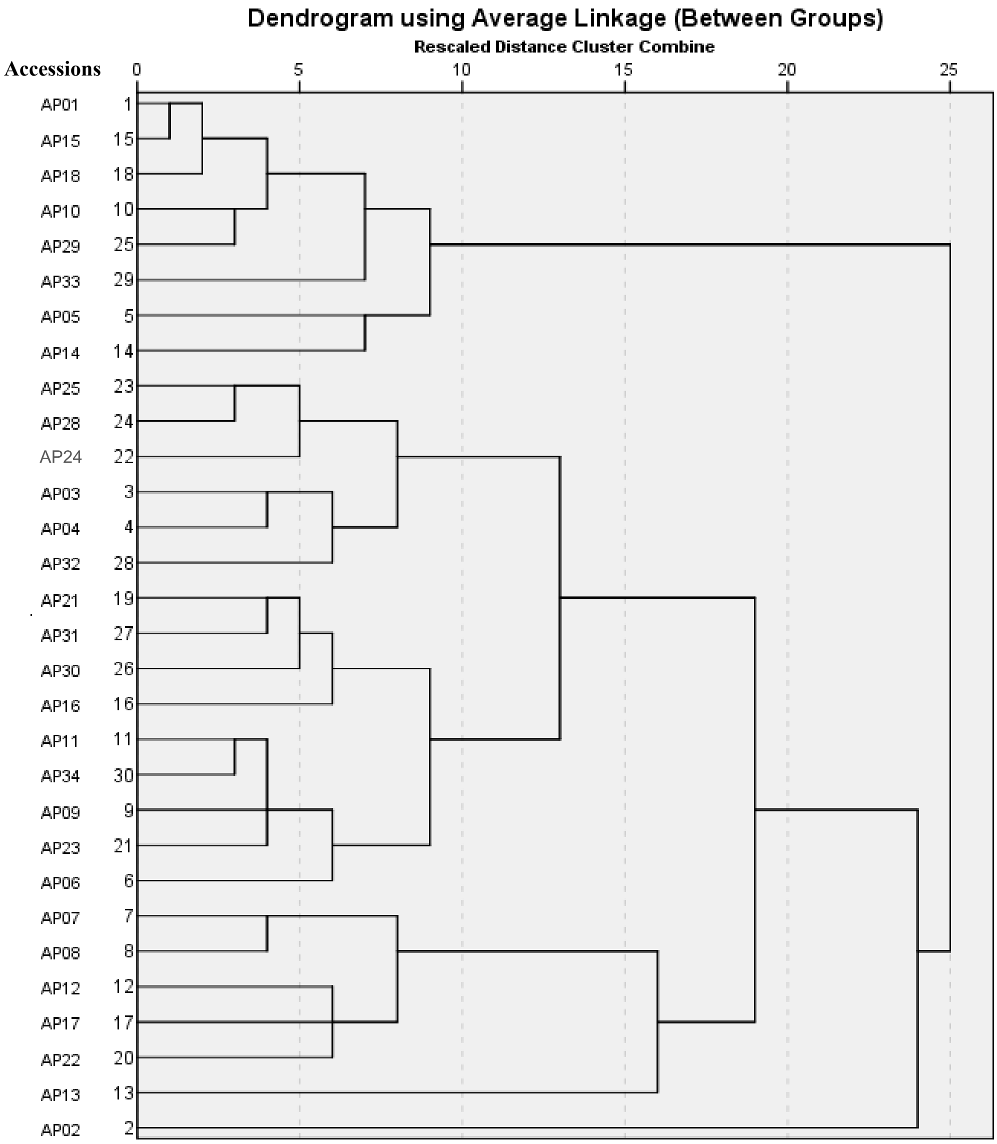

Figure 1 Phylogenetic tree manifesting the diversity among 30 accessions of Asiatic pennywort based on Euclidean distance coefficient identified by morphological and agronomical characteristics.

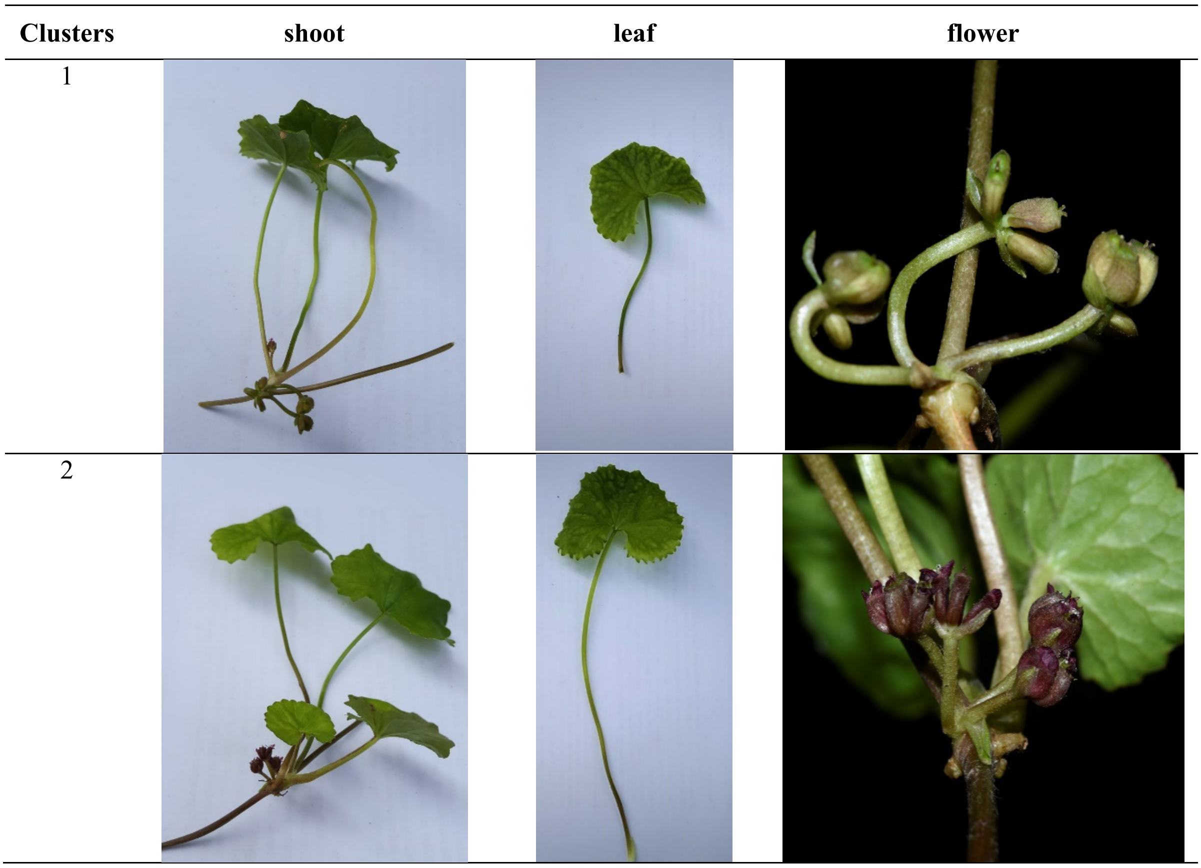

Figure 3 leaf and flower diversity in different 2 clusters of Asiatic pennywort in Thailand.

Table 4 Minimum, maximum, mean value and standard deviation (SD) of 11 agronomic characteristics of the 2 clusters of Asiatic pennywort.

Traits	Cluster 1				Cluster 2			
	min	max	mean	SD[1/]	min	max	mean	SD[1/]
1. plant height (cm.)	2.00	10.50	5.43	2.40	2.50	15.00	8.01	2.92
2. total leaf number	1.00	37.00	9.95	9.34	2.00	92.00	32.33	20.49
3. shoot number	1.00	14.00	3.83	3.53	1.00	40.00	11.84	7.78
4. leaf per shoot	1.00	5.00	2.62	1.02	0.83	7.25	2.90	0.94
5. leaf length (cm.)	1.00	6.20	2.92	1.26	2.00	8.50	4.61	1.38
6. leaf width (cm.)	0.80	3.80	1.90	0.74	1.00	6.00	3.02	1.00
7. Petiole length (cm.)	1.30	9.40	4.49	2.08	2.00	16.00	6.97	2.68
8. stolon number	0.00	4.00	1.02	1.15	0.00	13.00	3.23	2.24
9. stolon length (cm.)	0.00	40.00	13.04	14.56	0.00	125.50	53.44	27.11
10. panicle number	0.00	14.00	2.69	4.18	0.00	150.00	11.40	14.91
11. SPAD index	0.00	49.00	16.74	10.11	2.30	39.10	22.46	7.57

The research of different landraces and accessions variation are important for improvement and plant breeding strategy. Cluster analysis based on morphologies and agronomic characteristics was studied in various plant species, such as pigeon pea, rice and soybean [20-22]. In Asiatic pennywort, Chandrasekara *et al*. [17] was clustered the 14 morphologies of 5 morphotypes of Asiatic pennywort in Sri Lanka, the results revealed that 2 differed clusters were classified. Ravi *et al*. [23] identified the variability of 39 accessions of Asiatic pennywort in India based on 14 morphological traits, the results shown wide variability. In the other hand, the classification of Asiatic pennywort based on chromosome and genetic diversity with DNA markers were studied. The results revealed 2 polyploidy complex as diploid (2n = 2x = 18) and tetraploid (2n = 4x = 36) of Asiatic pennywort in Madagascar [24]. Although, the information of phenotypic diversity of Asiatic pennywort was not closely related to the actives ingredients and genetic diversity. However, the results of this study revealed a wide range of variability of morphological characteristics among the 30 accessions of Asiatic pennywort in Thailand.

Correlation coefficient of 11 morphology and agronomic characteristic of Asiatic pennywort

The correlation coefficient of 30 accessions Asiatic pennywort based on 11 morphology and agronomic characteristics were investigated (**Table 5**). The result revealed that plant height was highly significant positive correlated with petiole length (0.803). Total leaf number was highly significant positive correlated with the total leaf number, stolon number and stolon length (0.935, 0.791 and 0.710, respectively). Shoot number was highly significant positive correlated with stolon number and stolon length (0.758 and 0.721, respectively) and negatively correlated with leaf per shoot (–0.145). While, leaf length was highly significant positive correlated with leaf width and petiole length (0.896 and 0.789, respectively). Leaf width were highly significant positive correlated with petiole length (0.783). The result of stolon number was highly significant positive correlated with stolon length (0.706).

Table 5 Correlation coefficient on morphological and agronomical characteristic diversity of 30 accessions of Asiatic pennywort in Thailand.

Traits	Plant height	Total leaf number	Shoot number	Leaf per shoot	Leaf length (cm.)	Leaf width (cm.)	Petiole length (cm.)	Stolon number	Stolon length (cm.)	Panicle number	SPAD index
plant height (cm.)	1										
total leaf number	0.409**	1									
shoot number	0.340**	0.935**	1								
leaf per shoot	0.142	0.098	–0.145	1							
leaf length (cm.)	0.657**	0.459**	0.415**	0.220**	1						
leaf width (cm.)	0.657**	0.449**	0.387**	0.212**	0.896**	1					
Petiole length (cm.)	0.803**	0.352**	0.305**	0.175*	0.786**	0.783**	1				
stolon number	0.461**	0.791**	0.758**	0.026	0.493**	0.500**	0.389**	1			
stolon length (cm.)	0.537**	0.710**	0.721**	–0.044	0.590**	0.583**	0.535**	0.706**	1		
panicle number	0.305**	0.475**	0.475**	–0.017	0.339**	0.322**	0.298**	0.577**	0.443**	1	
SPAD index	0.195*	0.399**	0.368**	0.146	0.376**	0.365**	0.202*	0.351**	0.392**	0.163*	1

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

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

The principal component analysis was performed using 11 morphological and agronomical characteristics (**Table 6** and **Figure 2**). The result revealed that only 3 principal components (PCs) exhibited more than 1.0 Eigen values and showed about 86.028 % total variability among the characters were studied. The scree plot used to explain the percentage of variance associated with each principal component. The graph shown the highest variability in PC1 in comparison to the other 10 PCs. It indicated that the accessions selected from PC1 might be useful for Asiatic pennywort breeding program.

The results of this study shown that the morphological and agronomical characteristics of Asiatic pennywort closely related more characters. Chomicki *et al.* [25] reported the Asiatic pennywort stolon was emerged from the oldest leaf axils. While, stolon not only produced new leafy sympodial shoots at each node but also an axillary inflorescence. This indicated that the leafy accession might be produced more stolon, shoot and new leaf faster than accessions that few leaves. While, Aziz *et al*. [26] reported that Asiatic pennywort leaves contain the highest content of triterpenoids, asiaticoside and madecassoside than root and petiole. Also, the phenotype with smooth leaf has the higher composition of asiaticoside and madecassoside than the fringed leaf. It might be indicated that the morphological characteristic with highly related to leaf traits could be used for selection criteria in Asiatic pennywort breeding program.

Table 6 Eigen values, variance percentage and cumulative Eigen values percentage of Asiatic pennywort.

Traits	Principle components	Eigen values	% of Variance	Cumulative %
Plant height (cm.)	PC 1	6.611	60.103	60.103
Total leaf number	PC 2	1.628	14.804	74.907
Shoot number	PC 3	1.223	11.122	86.028
Leaf per shoot	PC 4	0.484	4.403	90.432
Leaf length (cm.)	PC 5	0.430	3.910	94.342
Leaf width (cm.)	PC 6	0.226	2.059	96.400
Petiole length (cm.)	PC 7	0.183	1.659	98.060
Stolon number	PC 8	0.089	0.813	98.873
Stolon length (cm.)	PC 9	0.058	0.524	99.397
Panicle number	PC 10	0.051	0.465	99.862
SPAD index	PC 11	0.015	0.138	100.000

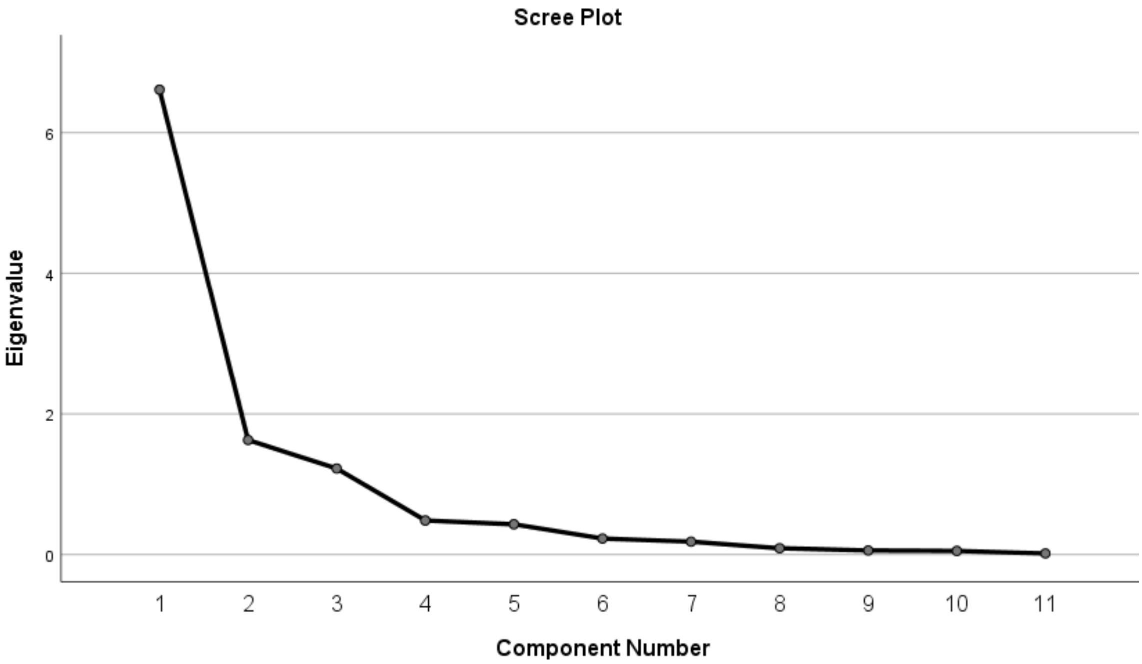

Figure 2 Scree plot of principal component analysis of Asiatic pennywort between eigen value and principal components.

Conclusions

The variability based on morphological and agronomic characteristics of Asiatic pennywort 30 accessions that collected from various parts of Thailand. The great variability was mainly observed in quantitative characteristics such as, leaf number, leaf length, leaf width, shoot number and stolon number are suitable for Asiatic pennywort germplasm characterization. Cluster analysis based on morphological and agronomic characteristics revealed that the Asiatic pennywort were classified into 2 differed clusters, which cluster 2 shown the highest mean value in the most characters. It indicated that accessions member in cluster 2 growth rapidly and high biomass accumulation. The Asiatic pennywort accessions in cluster 2 could be used for breeding program in the future.

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