

CHAPTER IV

RESULTS

1. Study on the geographic distribution of *P. mirifica* plants

1.1. Geographic distribution study

A survey of *P. mirifica* plants in natural habitats was carried out in some provinces from the three principal regions of Thailand during 1998-2000. It was at Amphur Doi Tao and Chiang Dao in Chiangmai province, Amphur Mae Sai in Chiangrai province, Amphur Kho Ka in Lumpang and Amphur Muang in Prae province, Amphur Tha Song Yang in Tak province (northern part), Amphur Waritchaphum, Sakonnakorn province and Amphur Phu Khieo in Chaiyaphum province, Amphur Pak Chong, Nakornratchasima (north-eastern part), Amphur Sai Yok, Tha Sao, Thong Pha Phum in Kanchanaburi province and Amphur Phra Phuttabat in Saraburi province (Central part) (**Figure 2**).

The geography of each study site is shown in **Figure 2** and **Table 3**.

The summarized of the area which *P. mirifica* could be found are as follows;

| | |
|--------------|--------------------------------------|
| Latitude | 13°43'-22°30' |
| Longitude | 98°15'-103°42' |
| Altitude | 78 – 393 meter above sea level |
| Topography | mountainous, terraces, hill, rolling |
| Soil Textile | loamy, sandy, gravel and limestone |

The highest elevation was 393 meters above the sea level at Amphur Ching Dao in Chiangmai province and the lowest elevation was 78 meters above the sea level at Amphur Tha Sao in Kanchanaburi province.

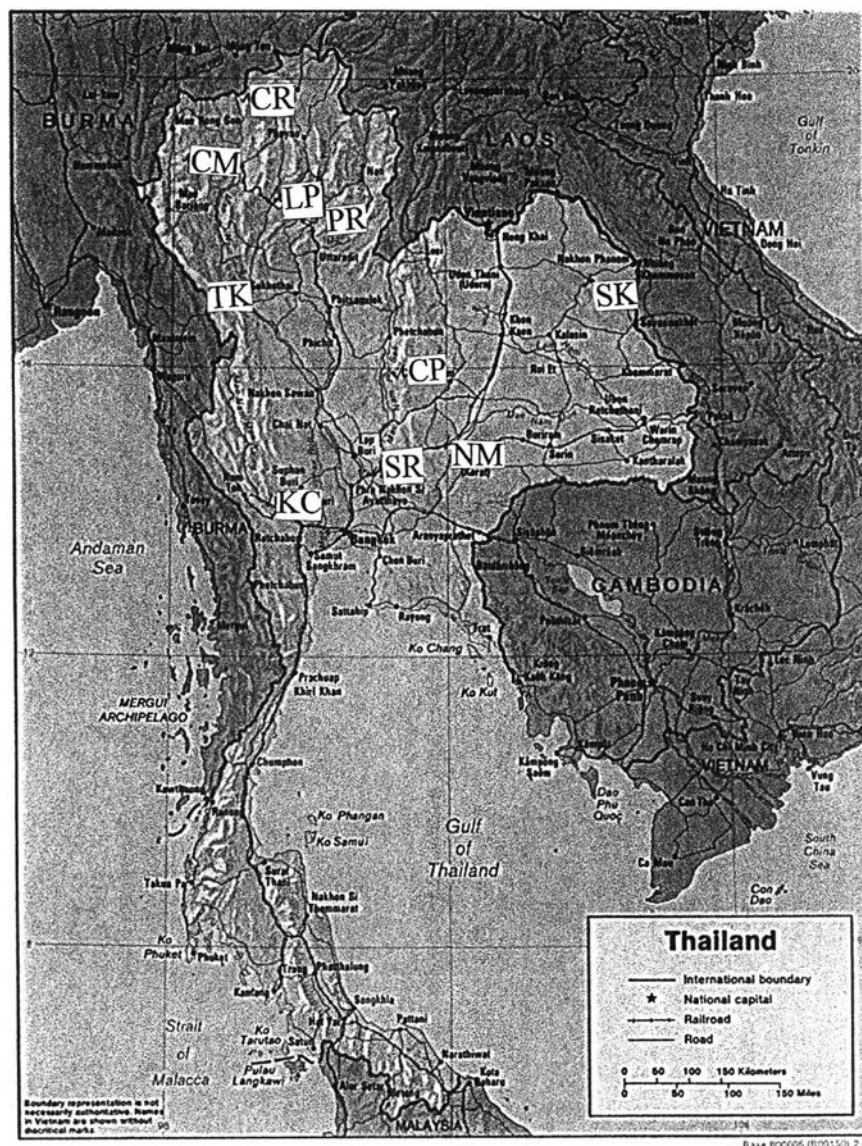


Figure 2 Map of the provinces that *P. mirifica* had been collected. It was at Chiangmai (CM), Chingrai (CR) Lumpang (LP), Prae (PR), Tak (TK) Sakonnakorn (SK), Chaiyaphum (CP), Nakornratchsima (NM), Kanchanaburi (KC) and Saraburi (SR) province.

Table 3 Geography of the sites under studies during 1998-2000.

| Site | Latitude | Longitude | Altitude | Topography | Soil Textile |
|-----------------------|-------------------|---------------------|----------|---------------|----------------------|
| 1. Northern part | | | | | |
| 1.1 Doi Tao, CM | 17° 54' - 18° 59' | 98° 35' - 98° 41' | 312 | Mountainous | Gravel |
| 1.2 Chaing Dao, CM | 19° 14' - 19° 28' | 98° 40' - 19° 00' | 393 | Mountainous | Gravel and limestone |
| 1.3 Mae Sai, CR | 19° 50' - 22° 30' | 99° 50' - 100° 42' | 220 | Mountainous | Gravel and limestone |
| 1.3 Koh Ka, LP | 18° 10' - 18° 12' | 99° 22' - 99° 25' | 241 | Terraces | Sandy and loamy |
| 1.4 Muang, PR | 18° 07' - 18° 10' | 100° 66' - 100° 10' | 161 | Basin | Loamy |
| 1.5 Tah Song Yang, TK | 17° 08' - 17° 14' | 98° 15' - 98° 24' | 121 | Mountainous | Gravel and limestone |
| 2. Northeastern part | | | | | |
| 2.1 Waritchaphum, SK | 17° 09' - 17° 13' | 103° 32' - 103° 42' | 250 | Mountainous | Sandstone and loamy |
| 2.2 Phu Khieo, CP | 16° 31' - 16° 36' | 101° 29' - 101° 49' | 220 | Mountainous | Sandstone and loamy |
| 2.3 Pak Chong, NM | 14° 41' - 14° 43' | 101° 14' - 101° 25' | 320 | Hill, Rolling | Sandstone and loamy |

CM = Chiangmai, LP = Lumpang, PR = Prae, KC = Kanchanaburi, TK = Tak, SK = Sakonnakorn, CP = Chaiyaphum, NM = Nakornratchasima, SR = Saraburi province and * = Field plot at Amphur Ban Pong, Ratchaburi province

Table 2 (continued)

| Site | Latitude | Longitude | Altitude | Topography | Soil Texture |
|------------------------|-------------------|---------------------|----------|---------------|----------------------|
| 3. Central part | | | | | |
| 3.1 Sai Yok, KC | 14° 08' - 14° 39' | 98° 35' - 99° 07' | 109 | Terraces | Gravel and limestone |
| 3.2 Tha Sao, KC | 14° 04' - 14° 09' | 99° 16' - 99° 38' | 92 | Terraces | Gravel and limestone |
| 3.3 Thong Pha Phum, KC | 14° 00' - 14° 34' | 99° 00' - 99° 16' | 119 | Mountainous | Gravel and limestone |
| 4.1 Phra Phuttabat, SR | 14° 40' - 14° 44' | 100° 47' - 100° 54' | 100 | Hill, Rolling | Limestone and loamy |
| *Ban Pong, RB | 13° 40' - 13° 47' | 99° 43' - 99° 52' | 78 | Floodplain | Loamy |

CM = Chiangmai, LP = Lumpang, PR = Prae, KC = Kanchanaburi, TK = Tak, SK = Sakonnakorn, CP = Chaiyaphum, NM = Nakornratchasima, SR = Saraburi province and * = Field plot at Amphur Ban Pong, Ratchaburi province

1.2 Description of *P.mirifica* in natural habitats

At Chiangmai and Kanchanaburi province, a lot of *P. mirifica* was found. The distribution was scattered which could be commonly seen along the side of the road from Amphur Tha Sao - Sai Yok - Thong Pha Phum in Kanchanaburi province and Amphur Chiang Dao - Chai Pra Karn and Doi Tao in Chaingmai. Meanwhile the plant in other sites was found smaller in distribution area.

The co-habitated plant of *P. mirifica* was typically bamboo. The plant was not be found in the forest consisted of high density trees. The vine of *P. mirifica* elongated for climbing over the trees while in an open area, the plant spread on the ground (**Figure 3**).

The stem was varied in sizes depending on age. The diameter in some plants was nearly 10 cm. The tuberous root had various shapes as shown in **Figure 4**. **Figure 5** showed the cross section with the year rings. During January - April, these were plants were flowering which could be seen clearly if it was the dominant plant in that area. The florescences had a various length approximately 10 – 100 cm (**Figure 6**). The florescence of the Chiangmai province collected plant was longer than that found in Kanchanaburi province. The florescence mature into pod with seed. The maturation were at the end of March to April. The flower of Chiangmai province differed from those of Kanchanaburi province. The petal from Chiangmai province showed a dark purple color while from

Kanchanburi showed purple-blue color (**Figure 7**). The seed number per pod (**Figure 8**) were varied between 1-10 seeds, the size was approximately 0.35 x 0.42 cm.



(A)



(B)

Figure 3 *P. mirifica* plants in natural habitat (A) Chiangmai, (B) Chaiyaphum, (C) Kanchanaburi and (D-E) Sakonnakorn province. An arrow indicated *P. mirifica* plants



(C)



(D)



(E)

Figure 3 *P. mirifica* plants in natural habitat (A) Chiangmai, (B) Chaiyaphum, (C) Kanchanaburi and (D-E) Sakonnakorn province. An arrow indicated *P. mirifica* plants. (continued)



Figure 4 The shape of tuberos roots of *P. mirifica* obtained from the sites

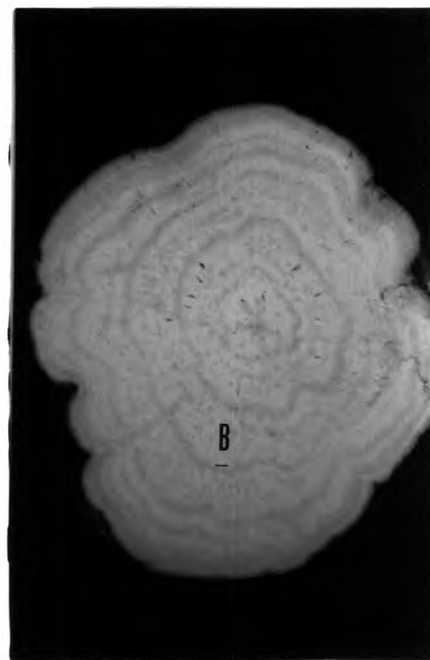


Figure 5 The cross section of *P. mirifica* tuberous roots (A) one year old plant (B) approximately eight years old plant



(A)



(B)

Figure 6 *P. mirifica* florescences collected from (A) Chiangmai and (B) Kanchanaburi province



(A)



(B)

Figure 7 The *P. mirifica* flower collected from (A) Chiangmai and (B) Kanchanaburi province

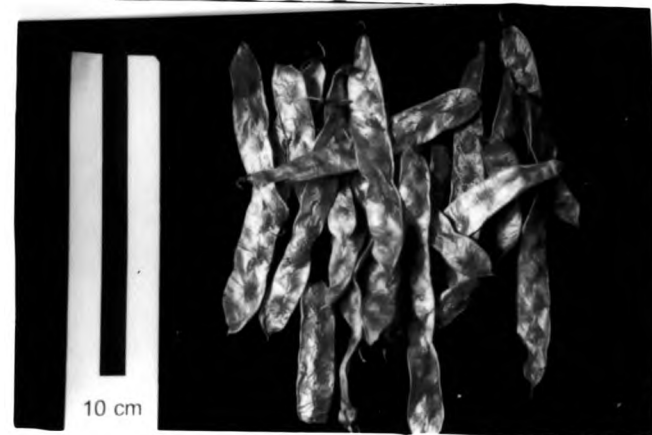


Figure 8 *P. mirifica* pods

The external characteristics of seeds were shown in **Table 4** and **Figure 9**. It was found that the seeds could be classified into 11 characters according to the shape, color and pattern of seed surface. It revealed that the seed type could be divided into 2 distinct shapes as bean and round shape. The bean shape was found in all varieties while the round shape could be found only in Amphur Doi Tao, Chiangmai province. The colors were varied as shading and could be defined into 4 main colors; dark brown, brown, brown - green and green. The seeds also appeared for the surface pattern as striation and pale. The green - pale -bean shape (D) and brown - striation - bean shape (E) seeds were commonly found in all samples.

Table 4 External characteristics of seeds collected during 1998-2000

| Sites | % Type of external characteristic | | | | | | | | | | |
|--------------------|-----------------------------------|----|---|----|----|----|----|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J | K |
| Doi Tao, CM | 10 | - | - | 8 | 31 | - | 37 | 5 | 3 | 4 | 2 |
| Tha Sao, KC | 15 | - | - | 11 | 57 | - | 17 | - | - | - | - |
| Thong Pha Phum, KC | 24 | 38 | - | 8 | 15 | 15 | - | - | - | - | - |
| Sai Yok, KC | 5 | 40 | - | 6 | 3 | 28 | 4 | - | - | - | - |
| Tha Song Yang, TK | - | 42 | - | 10 | 16 | - | 32 | - | - | - | - |
| KC in field trial | 23 | 6 | 1 | 1 | 16 | 8 | 53 | - | - | - | - |

CM = Chiangmai province, KC = Kanchanaburi province, TK = Tak province

Each letter is presented the type of character as follows;

A = Bean shape + Pale + Dark brown

B = Bean shape + Pale + Brown

C = Bean shape + Pale + Brown-green

D = Bean shape + Pale + Green

E = Bean shape + Striation + Brown

F = Bean shape + Striation + Brown-green

G = Bean shape + Striation + Green

H = Round shape + Pale + Brown

I = Round shape + Pale + Green

J = Round shape + Striation + Brown

K = Round shape + Striation + Green

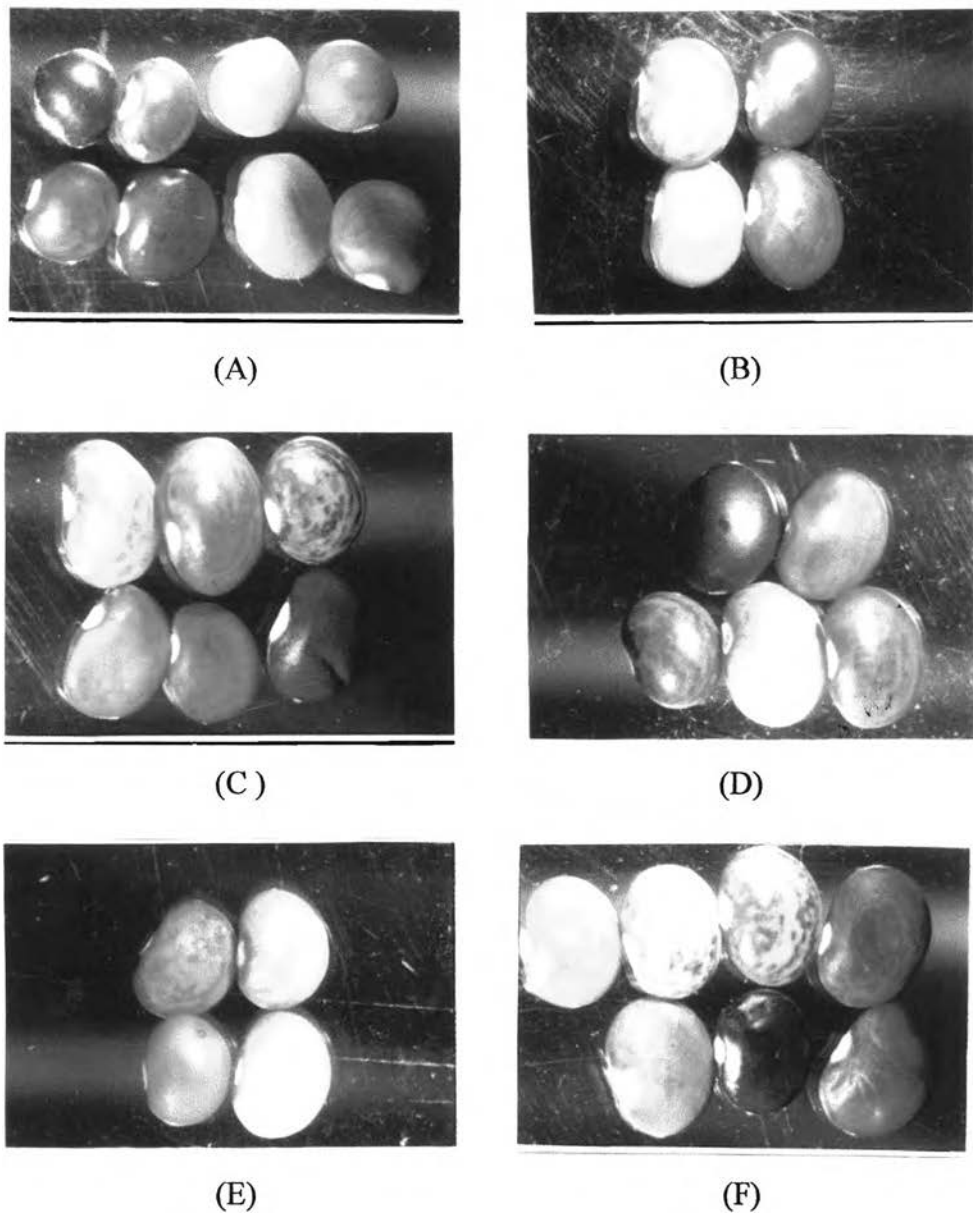


Figure 9 The external characteristics of seeds could be classified into 11 characteres according to the shape, color and surface pattern. The seeds were collected from (A) Amphur Doi Tao, Chiangmai (B) Amphur Tha Sao, (C) Amphur Thong Pha Phum, (D) Amphur Sai Yok, Kanchanaburi, (E) Amphur Tha Song Yang, Tak province and (F) field grown F1 plant

1.3 The TLC analysis

1.3.1 Qualitative analysis : general TLC finger print of *P. mirifica*

TLC method was submitted for the qualitative analysis of the crude chemical contents of *P. mirifica* tuberous root. The methanolic crude extract of each variety was prepared as described in chapter II.

The composition of chloroform: methanol in the ratio 90:10 was carried out as a solvent system for separation of some chemicals in the tuberous root powder.

Figure 10 shows the total bands of *P. mirifica* tuberous extracts collected from various places. At 254 nm, the R_f values varied from 0.09-0.80 which consisted of the twelve bands as shown in **Table 5**. Descending the number of R_f value that was found in various samples as shown in **Table 6**. The band with R_f value that was found in various samples as shown in **Table 6**. The band with R_f value of 0.43 and 0.45 which appeared purple were found common. At 365 nm, the R_f values varied from 0.07-0.76 as shown in **Table 6**. The R_f value of 0.40, 0.43 and 0.51 were found in nine out of ten samples (**Table 7**).

It also revealed that each sample contained various bands from 4-12 bands which was obtained under UV light 254 nm and 6-12 bands under visible light 365 nm. At 365 nm, the samples which were collected from Amphur Doi Tao and Chiang Dao, Chiangmai varieties showed the same set of bands.

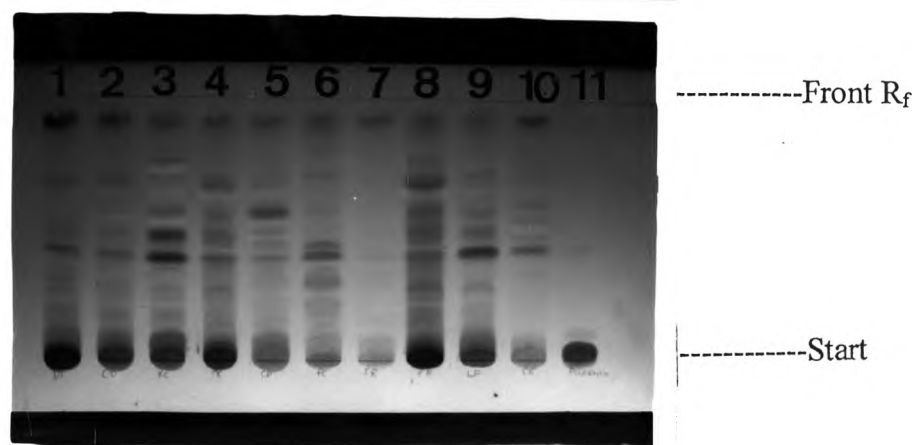


Figure 10 TLC patterns of *P. mirifica* tuberous root extracts using solvent system: chloroform: methanol (90:10) under UV light at 254 nm. Each collected sample is labeled as follows;

| | |
|---------------------------------|-----------------------------|
| 1 = Doi Tao, Chiangmai | 7 = Phra Phuttaba, Saraburi |
| 2 = Chiang Dao, Chaingmai | 8 = Muang, Prae |
| 3 = Sai Yok, Kanchanburi | 9 = Koh Ka, Lumpang |
| 4 = Waritchaphum, Sakonnakorn | 10 = Mae Sai, Chiangrai |
| 5 = Phu Khieo, Chaiyaphum | 11 = Standard puerarin |
| 6 = Pak Chong, Nakornratchasima | |

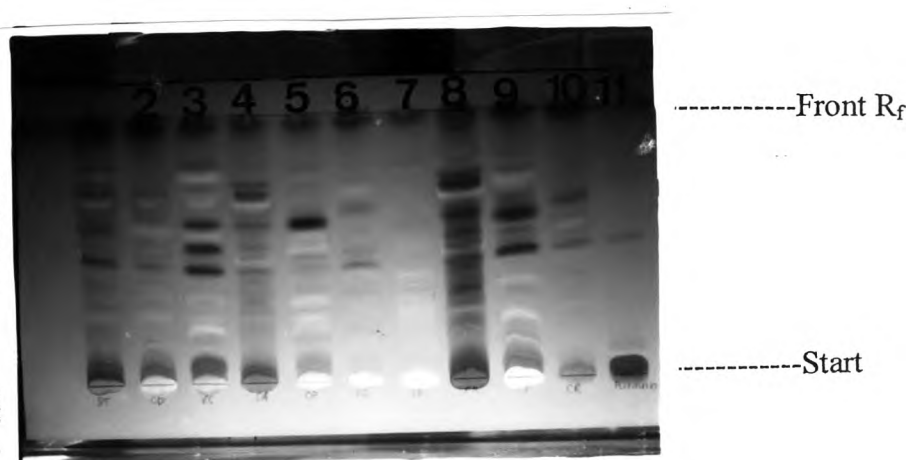


Figure 11 TLC patterns of *P. mirifica* tuberous root extracts using solvent system: chloroform: methanol (90:10) under visible light at at 365 nm. Each collected sample is labeled as follows;

- | | |
|---------------------------------|-----------------------------|
| 1 = Doi Tao, Chiangmai | 7 = Phra Phuttaba, Saraburi |
| 2 = Chiang Dao, Chaingmai | 8 = Muang, Prae |
| 3 = Sai Yok, Kanchanburi | 9 = Koh Ka, Lumpang |
| 4 = Waritchaphum, Sakonnakorn | 10 = Mae Sai, Chiangrai |
| 5 = Phu Khieo, Chaiyaphum | 11 = Standard puerarin |
| 6 = Pak Chong, Nakornratchasima | |

Table 5 The R_f values and color of TLC pattern obtained from *P. mirifica* tuberous root crude extract from different provinces under UV light 254 nm

| Color / R _f | Purple-white | Yellow | Light purple | Dark purple | Light purple | Dark purple | Light purple | Orange | Light purple | Blue | Light purple | Dark purple |
|------------------------|--------------|--------|--------------|-------------|--------------|-------------|--------------|--------|--------------|------|--------------|-------------|
| Sample | 0.09 | 0.16 | 0.29 | 0.43 | 0.45 | 0.47 | 0.53 | 0.56 | 0.63 | 0.64 | 0.71 | 0.80 |
| DT | / | - | / | / | / | / | / | - | / | / | / | - |
| CD | / | - | / | / | / | / | / | - | / | / | - | - |
| KC | / | / | / | / | / | / | - | / | / | - | / | / |
| SK | / | - | / | / | / | / | - | / | / | / | - | - |
| CP | / | - | / | / | / | - | / | / | / | / | - | / |
| PC | / | - | - | / | / | / | - | - | / | - | - | - |
| SR | / | - | / | / | / | - | - | - | - | - | / | - |
| PR | / | / | / | / | / | / | - | / | / | / | - | / |
| LP | / | / | / | / | / | / | / | / | / | / | / | / |
| CR | - | - | / | / | / | - | - | / | - | - | - | - |
| Total | 9 | 3 | 9 | 10 | 10 | 8 | 4 | 6 | 8 | 6 | 4 | 4 |

DT = Doi Tao and CD = Chiang Dao, Chiangmai province, KC = Kanchanaburi, SK = Sakonnakorn, CP = Chaiyaphum, PC = Pak Chong, Nakornratchasim, SR = Saraburi, PR = Prae, LP = Lumpang and CR = Chiangrai province

Table 6 The R_f values and color of TLC pattern obtained from *P. mirifica* tuberous root crude extract from different provinces under visible light 365 nm

| Color / R _f | White | White | Yellow | Green | Purple | Green | Yellow | White | Green | Purple | Green | Orange | Purple | Green | White |
|------------------------|-------|-------|--------|-------|--------|-------|--------|-------|-------|--------|-------|--------|--------|-------|-------|
| Sample | 0.07 | 0.16 | 0.20 | 0.20 | 0.23 | 0.27 | 0.28 | 0.35 | 0.40 | 0.43 | 0.51 | 0.56 | 0.61 | 0.73 | 0.76 |
| DT | / | / | - | - | / | / | - | - | / | / | / | - | / | / | - |
| CD | / | / | - | - | / | / | - | - | / | / | / | - | / | / | - |
| KC | / | / | / | - | / | / | - | - | / | / | / | / | / | / | / |
| SK | / | / | - | / | / | - | - | / | / | / | / | / | / | / | - |
| CP | - | / | / | - | / | - | / | - | / | / | / | / | / | / | - |
| PC | - | - | - | - | / | / | - | - | / | / | / | / | - | / | - |
| SR | - | - | - | - | - | / | - | - | - | - | - | - | - | - | - |
| PR | / | - | - | / | / | / | - | / | / | / | / | / | / | - | / |
| LP | / | - | / | - | / | / | - | - | / | / | / | / | / | - | / |
| CR | - | - | / | - | - | - | / | - | / | / | / | / | - | - | - |
| Total | 6 | 5 | 4 | 2 | 8 | 7 | 2 | 2 | 9 | 9 | 9 | 7 | 7 | 6 | 3 |

DT = Doi Tao and CD = Chiang Dao, Chiangmai province, KC = Kanchanaburi, SK = Sakonnakorn, CP = Chaiyaphum, PC = Pak Chong, Nakornratchasima, SR = Saraburi, PR = Prae, LP = Lumpang, CR = Chiangrai province

Table 7 The numbers of Rf values obtained from *P. mirifica* tuberous root crude extract from ten different places under UV-visible light at 254 and 365 nm

| No. of samples | Rf value | |
|----------------|------------|--------------------|
| | 254 nm | 365 nm. |
| 10 | 0.43, 0.45 | - |
| 9 | 0.09, 0.29 | 0.40, 0.43, 0.510. |
| 8 | 0.47, 0.63 | 0.23, 0.76 |
| 7 | - | 0.27, 0.65, 0.61 |
| 6 | 0.56, 0.64 | 0.07, 0.73 |
| 5 | - | 0.16 |
| 4 | 0.71, 0.80 | 0.20 |
| 3 | 0.16 | 0.76 |
| 2 | - | 0.20, 0.28, 0.35 |
| 1 | - | - |

1.3.2 Quantitative analysis of puerarin in *P. mirifica* tuberous root by TLC desitometry

1.3.2.1 Setting up the separation method for puerarin using TLC method

The composition of chloroform-methanol-H₂O with the ratio of 40:40:2 was found to be the optimum solvent system for the separation of puerarin. Under these conditions, a blue band is clearly demonstrated (**Figure.11**). The R_f value was found to be approximately 0.70.

It was found that the absorption spectra of puerarin in the crude extract were similar to the standard puerarin as shown in **Figure 12**.

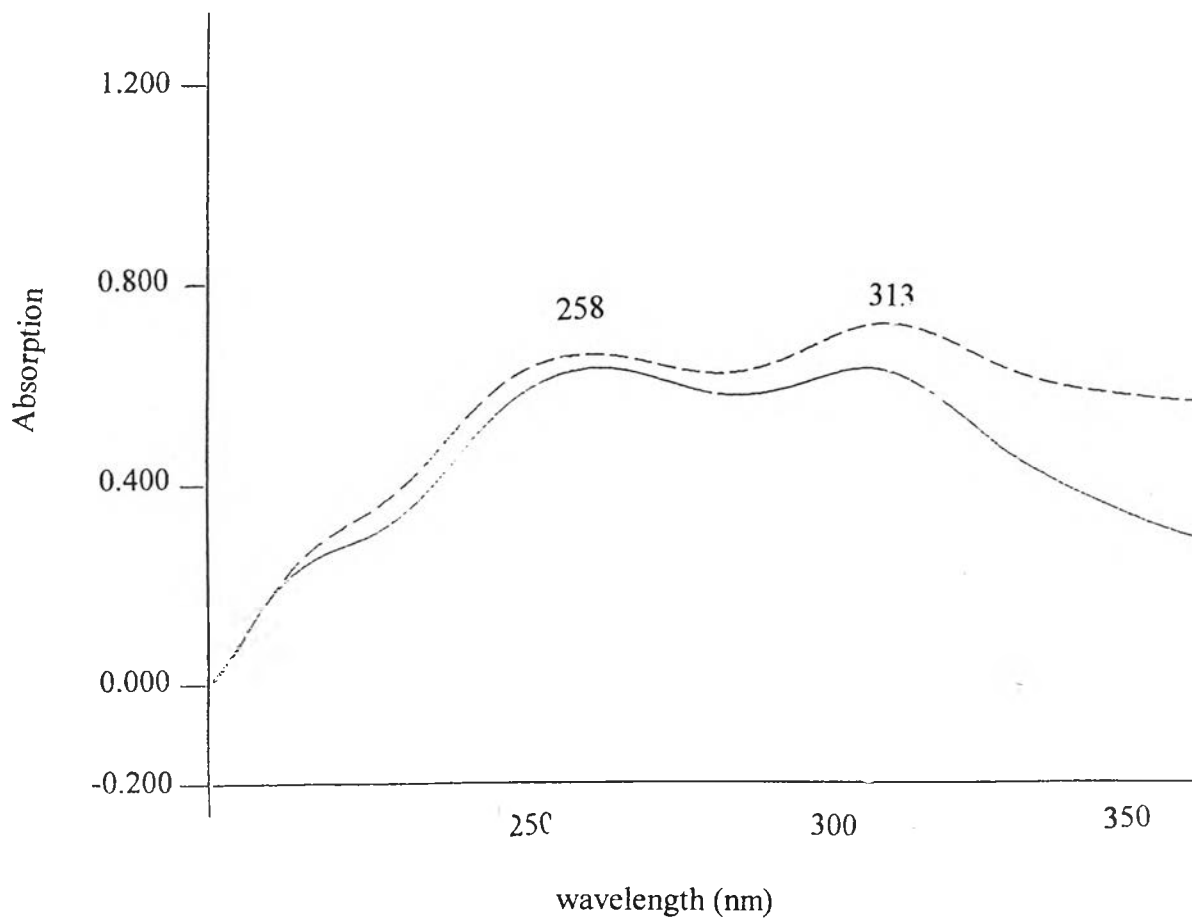


Figure 12. UV-light absorption spectra of puerarin (—) from the crude extract and standard puerarin (-----) in methanol by Dual -Wave length TLC-scanner. The maximum absorption wavelength was found to be at 258 and 313 nm.

1.3.2.2 Standard curve of puerarin

The relationship between concentrations of puerarin and peak areas of the bands were obtained in the range of 0.97-15.63 mg/ml which was established as the linear graph in **Figure 13**, a submitted equation was ; $y = 8166.2x + 8974.9$ which the correlation coefficient (r) was 0.9981 and $R^2 = 0.9961$

Let y = Peak area
 x = Concentration of puerarin (mg/ml)
 r = Correlation coefficient

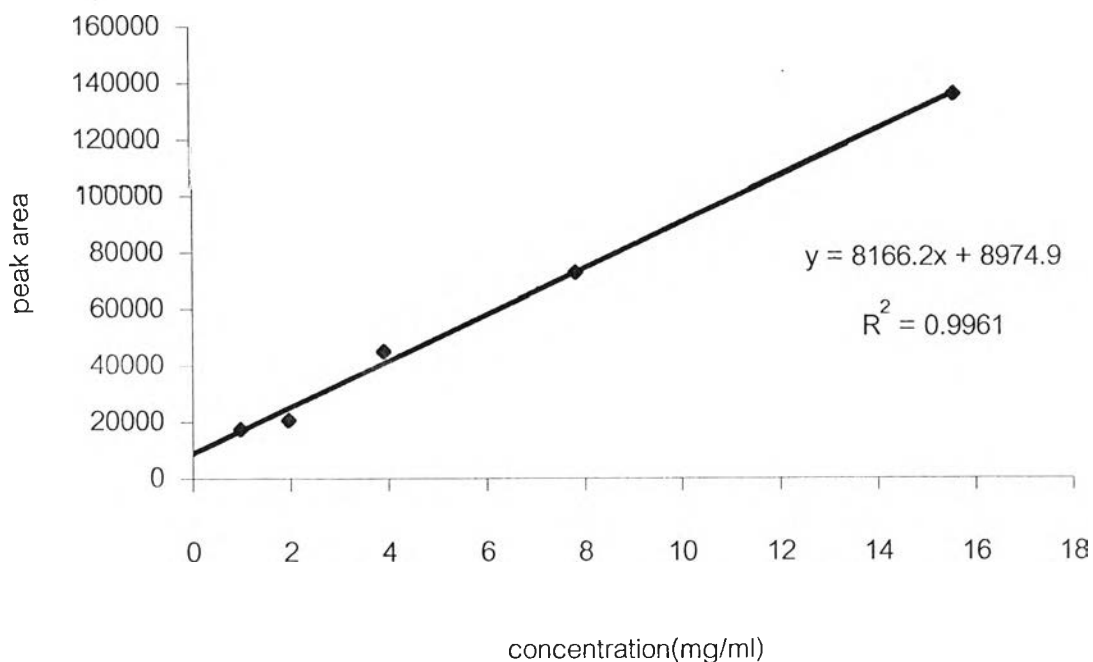


Figure 13 Standard curve of puerarin established by TLC-densitometry method

1.3.2.3 The quantitative analysis of the extracted puerarin from *P. mirifica* varieties

Based on the TLC method, puerarin from the crude extract was quantified from each sample derived from Chaingmai (CM), Chiangrai (CR), Lumpang (LP), Prae (PR), Sakonnakorn (SK), Chaiyaphum (CP), Nakornratchasima (NM), Kanchanburi (KC) province. The amount of puerarin in each sample is shown in **Table 7** and **Figure 14**.

All of the tuberous roots from different places contained variable amount of puerarin. The collected sample of Sakonnakorn province contained the highest content of puerarin which was 3.7649 % w / w, the lowest was 0.6015 % w / w from the collected sample from Prae province.

Table 8 The percentages of puerarin in collected *P. mirifica* tuberous roots obtained by TLC densitometric method

| Sample | CD | DT | CR | LP | PR | SK | CP | PC | KC |
|----------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Puerarin | 1.2675 | 1.6658 | 0.5353 | 0.6332 | 0.3970 | 3.7649 | 0.6015 | 0.4298 | 2.5775 |
| % w / w | | | | | | | | | |

CD = Amphur Chiang Dao and DT = Doi Tao in Chiangmai province, CR = Chiangrai province, PR = Prae provine, LP = Lumpang province , SK = Sakonnakorn province , CP = Chaiyaphum province, PC = Amphur Pak Chong in Nakornratchasima province and KC = Kanchanaburi province

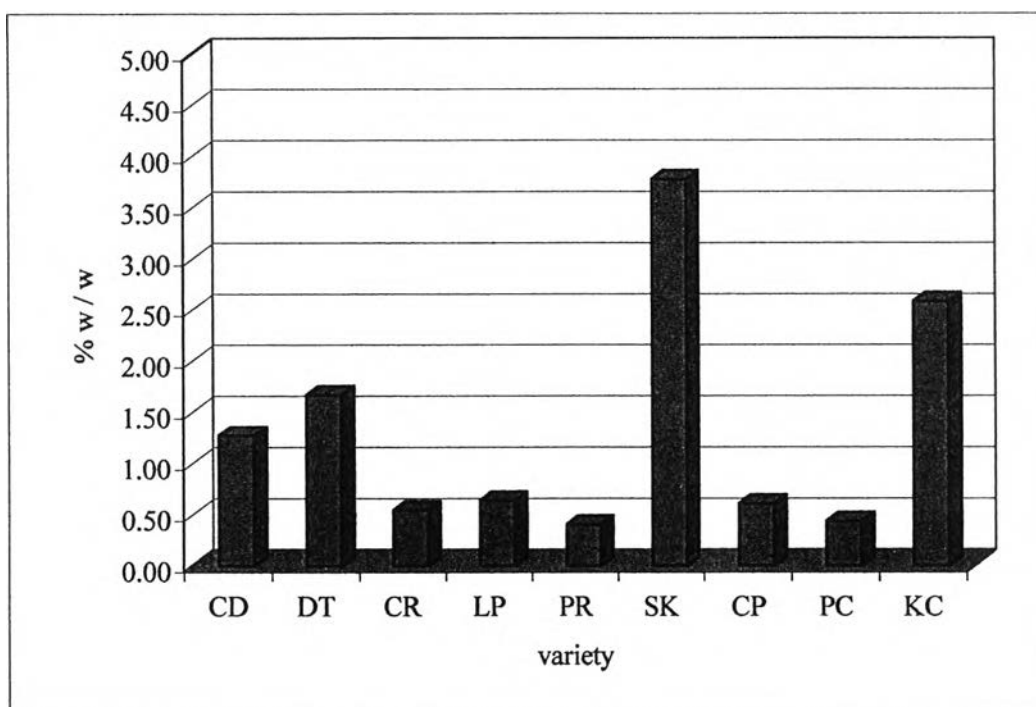


Figure 14 Puerarin contents in the collected *P. mirifica* tuberous roots from various places as follows; CD = Amphur Chiang Dao and DT = Doi Tao in Chiangmai province, CR = Chiangrai province, PR = Prae provine, LP = Lumpang province, SK = Sakonnakorn province, CP = Chaiyaphum province, PC = Amphur Pak Chong in Nakornratchasima province and KC = Kanchanaburi province

2. Study on the genetic differences of *P. mirifica* plants

2.1 The growth of the field trial F1 *P. mirifica*

The F1 *P. mirifica* plant of the Chiangmai and Kachanaburi variety could be grown in the field trail. The vine of the plant were laid on and spread over the ground. One plant may consist of more than one stem. The tuberous root could be produced from the internode that was laid on the ground and covered by the soil. One year old plant of both varieties could be flowering. The percentage of the plants which could be flowering of Chiangmai and Kanchanaburi variety were 4% and 7%, respectively. The mean of the length of the florescences of the Chiangmai variety was approximately 75 cm while that of the Kanchanaburi variety was approximately 65 cm. (**Table 9** and **Figure 15**). The difference of flower between Chiangmai and Kanchanaburi varieties was the color of the petal which the Chaingmai variety exhibited purple and blue while Kanchanaburi variety had lighter color (**Figure 15 (B)**). The flower of Chiangmai variety could not produced the pod and seed but could be found in three of seven plants of Kanchanaburi variety. The mean value of the pod length was approximately 7.5 cm. The shortest was 2 cm and the longest was 12 cm. The pod contained 2-6 seeds

Table 9 Flower characteristics of the field grown *P. mirifica* from the Chiangmai and Kanchanaburi variety

| Character | Chiangmai | Kanchanaburi |
|-----------------------------------|-----------|--------------|
| No. of flowering plant | 4% | 7% |
| Florescences length (cm) | 75 | 65 |
| Mean value of florescences number | 25 | 20 |



(A)

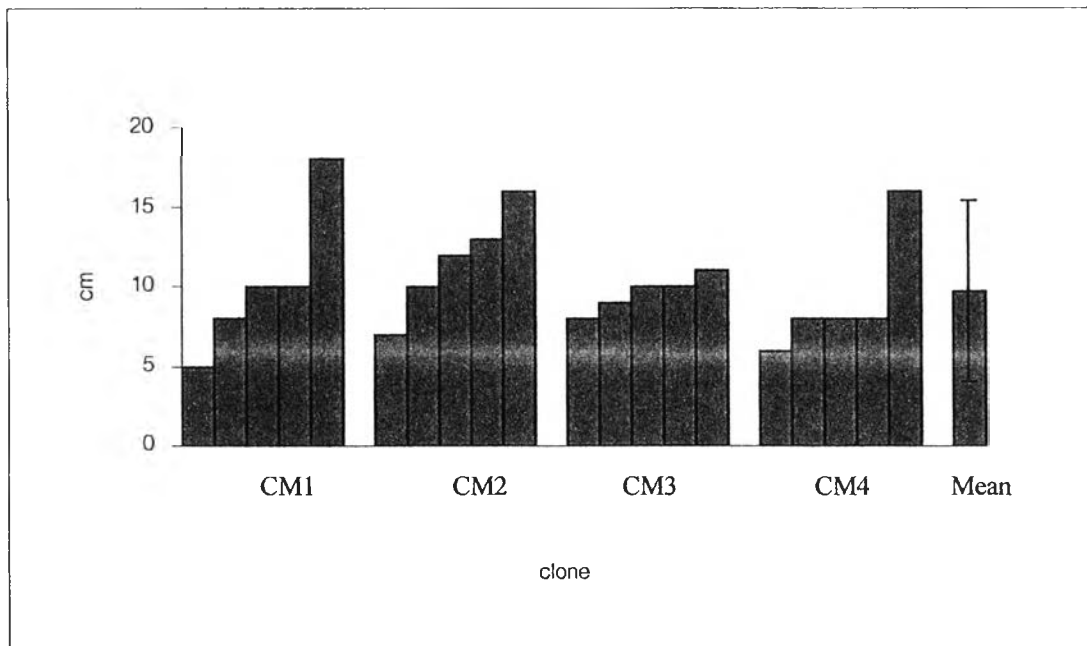


(1)

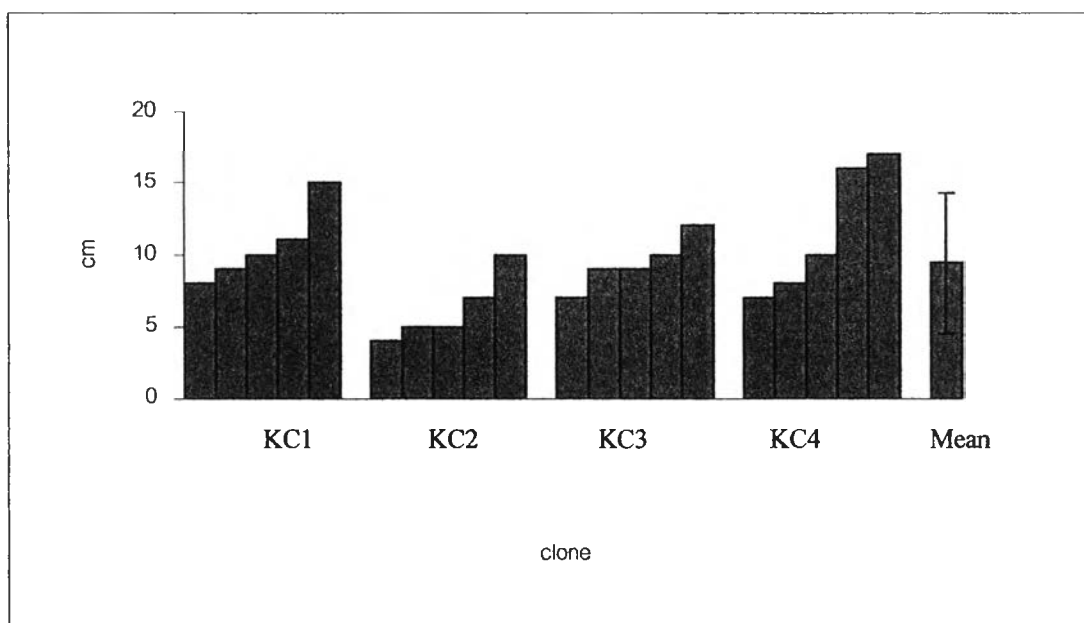
(2)

(B)

Figure 15 The florescences and flowers of the F1 field grown *P. mirifica* from (A and B(1)) the Chaingmai and (B(2)) Kanchanaburi variety

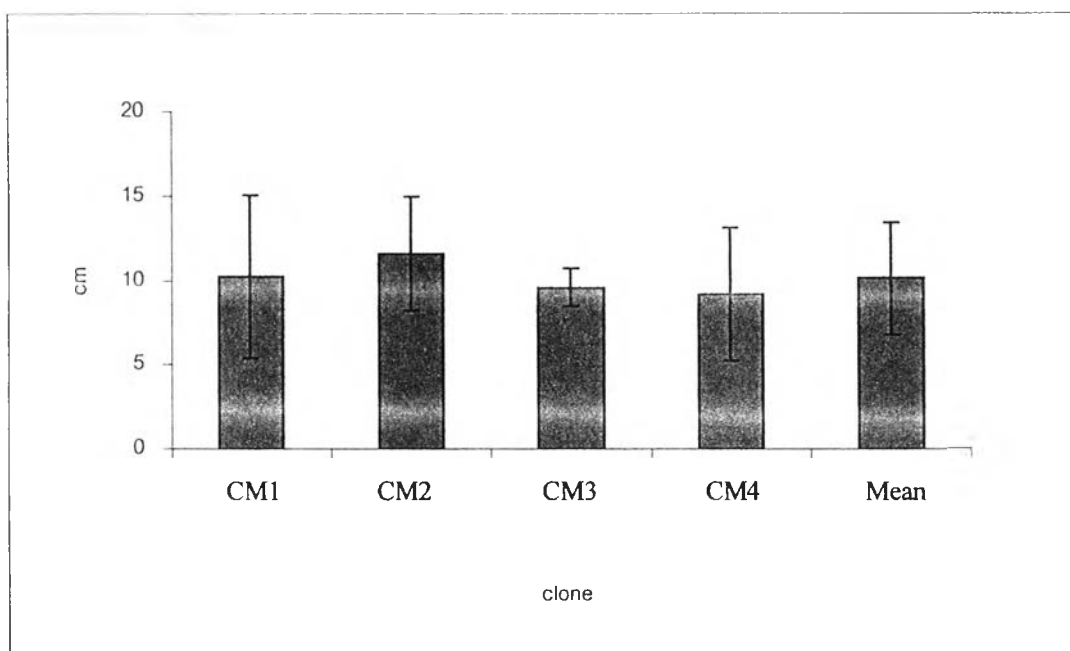


(A)

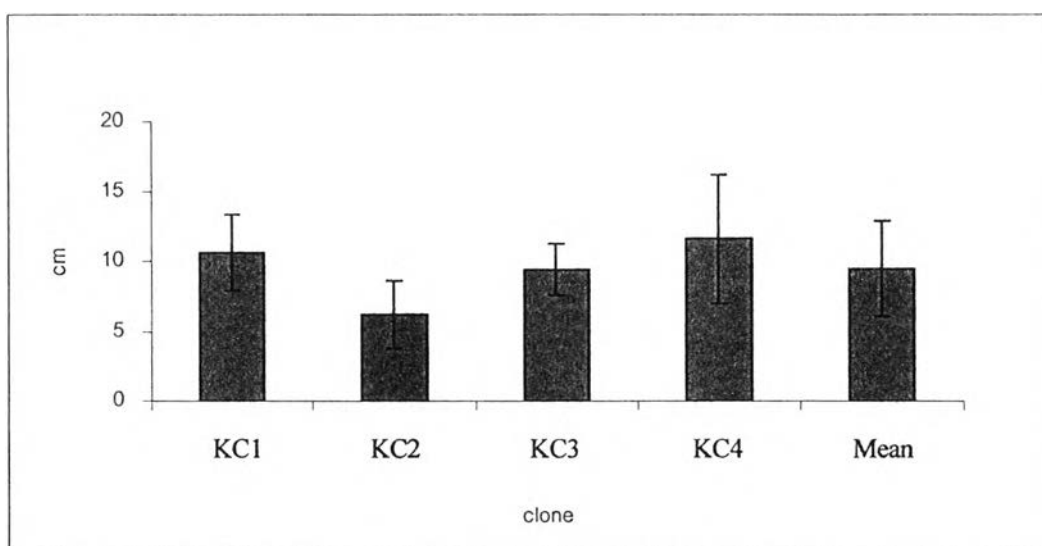


(B)

Figure 16 The peristeme lengths of the field grown *P. mirifica* from (A) the Chiangmai and (B) Kanchanaburi variety



(A)



(B)

Figure 17 The mean and SD value of peristem length of the field grown *P. mirifica* in (A) Chiangmai and (B) Kanchanaburi variety

2.2 The tuberous roots of the field grown F1 *P. mirifica* plants

The formation of tuberous root of F1 plants could be classified into four distinct shapes as sausage, flat, triangular and funnel – alike (**Figure 18 (D)**). It was found that the plant usually exhibited one main root which was the largest root. The lengths of the main roots are shown in **Table 11**. The maximum, minimum and mean \pm SE value of the length of main root were 150, 26 and 67.90 ± 34.14 cm for the Chiangmai variety, and 90, 10 and 47.60 ± 19.69 cm for the Kanchanburi variety, respectively. Comparison of mean value between the two varieties revealed the significant difference ($p < 0.05$), while no significant difference was found within clones.

Three out of twenty plants of each variety showed no formation of tuber but exhibited only adventitious roots (**Figure 18 (A)**.) Thus the weight of such roots was not recorded.

นโยบายของคณะวิทยาศาสตร์เกี่ยวกับการเผยแพร่วิทยานิพนธ์ของนิสิตบัณฑิตศึกษา

คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย มีนโยบายที่จะสนับสนุนให้มีการเผยแพร่วิทยานิพนธ์ ซึ่งเป็นส่วนหนึ่งของการศึกษาในระดับบัณฑิตศึกษา เพื่อส่งเสริมความก้าวหน้าทางวิชาการและเพื่อประโยชน์ต่อสาธารณชนโดยส่วนรวม

แบบแสดงความคิดเห็นของคณะกรรมการสอบวิทยานิพนธ์เกี่ยวกับการเผยแพร่วิทยานิพนธ์
ของนิสิตระดับบัณฑิตศึกษาคณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ชื่อผู้เสนอวิทยานิพนธ์ นางสาวรัตนา ปานเรียนแสน

เลขประจำตัว 4072485223

นิสิตชั้นปริญญา มหาบัณฑิต ภาควิชา -

สาขาวิชา เทคโนโลยีชีวภาพ

ชื่อเรื่องวิทยานิพนธ์

~~การกระจายและลักษณะของประชากรกวาวเครือขาว~~ ~~จากแหล่งต่างๆ~~
ในประเทศไทย

DISTRIBUTION AND CHARACTERIZATION OF Pueraria morifica
POPULATIONS FROM VARIOUS PARTS OF THAILAND

ชื่ออาจารย์ที่ปรึกษาวิทยานิพนธ์ รองศาสตราจารย์ ดร.วิชัย เชิดชูวิศวกรรม

คณะกรรมการสอบวิทยานิพนธ์ได้พิจารณาแล้ว มีความเห็นว่าวิทยานิพนธ์ฉบับนี้

1. ควรอนุญาตให้เผยแพร่ได้
2. ไม่ควรอนุญาตให้เผยแพร่วิทยานิพนธ์ โดย

2.1 ไม่กำหนดเวลา

2.2 สงวนสิทธิการเผยแพร่ตั้งแต่บัดนี้จนถึงวันที่ 26 เดือน สิงหาคม พ.ศ. 2553

โปรดระบุเหตุผลที่ไม่อนุญาตตามข้อ 2.1 หรือ 2.2

.....
.....

(ลงนาม)

ประธานกรรมการสอบวิทยานิพนธ์

26. 10. 53

หมายเหตุ ในกรณีข้อ 2 ให้อาจารย์ที่ปรึกษาวิทยานิพนธ์และนิตินำวิทยานิพนธ์ส่งให้บัณฑิตวิทยาลัยโดยตรง ทั้งนี้ เพื่อจะได้พิจารณาเหตุผลและตรวจแบบฟอร์มและรับเอกสารข้อดีตราไว้เป็นหลักฐาน



บันทึกข้อความ

ส่วนราชการ หลักสูตรเทคโนโลยีชีวภาพ คณะวิทยาศาสตร์ จุฬาฯ โทร.2185384

ที่ ศษ. ๕๕๑

วันที่ 9 ตุลาคม 2543

เรื่อง ขอส่งวิทยานิพนธ์ฉบับสมบูรณ์ล่าช้า

เรียน เลขาธิการบัณฑิตวิทยาลัย

- สิ่งที่ส่งมาด้วย
1. ต้นฉบับวิทยานิพนธ์ฉบับสมบูรณ์จำนวน 4 เล่ม
 2. แผ่นดิสเก็ตต์บรรจุข้อมูลวิทยานิพนธ์ 1 แผ่น และ Template ทาง
บรรณานุกรม 1 แผ่น

ด้วย น.ส.รัตนา ปานเรียนแสน รหัสประจำตัว 4072485723 นิสิตในหลักสูตรวิทยาศาสตรมหาบัณฑิต สาขาเทคโนโลยีชีวภาพ ได้ผ่านการสอบวิทยานิพนธ์แล้ว ในวันที่ 26 กันยายน 2543 ซึ่งกรรมการมีมติให้แก้ไขและเปลี่ยนแปลงชื่อหัวข้อวิทยานิพนธ์ ซึ่งจำเป็นต้องใช้เวลาในการตรวจแก้ ดังนั้นจึงใคร่ขอขยายเวลาการส่งต้นฉบับวิทยานิพนธ์ฉบับสมบูรณ์จากวันที่ 6 ตุลาคม พ.ศ.2543 เป็นวันที่ 9 ตุลาคม พ.ศ.2543

จึงเรียนมาเพื่อโปรดดำเนินการต่อไปด้วย จักเป็นพระคุณยิ่ง

(ผู้ช่วยศาสตราจารย์ ดร.วิเชียร ริมphanichkij)

รักษาการแทนเลขาธิการหลักสูตรเทคโนโลยีชีวภาพ

(รองศาสตราจารย์ ดร.วิชัย เชิดชูศาสตร์)

อาจารย์ที่ปรึกษาวิทยานิพนธ์

รัตนา ปานเรียนแสน:ลักษณะของประชากรกวาวเครือขาวจากแหล่งต่างๆ ของ
ประเทศไทย (CHARACTERIZATION OF *Pueraria mirifica* POPULATIONS
FROM VARIOUS PARTS OF THAILAND) อ.ที่ปรึกษา: รศ.ดร.วิชัย เชิดชู
ศาสตร์ 116 หน้า ISBN 974-346-702-5

การสำรวจการกระจายของกวาวเครือขาว (*Pueraria mirifica*) ใน 10 จังหวัดในภาค
เหนือ ภาคตะวันออกเฉียงเหนือ และภาคกลางของประเทศไทยระหว่างปี พ.ศ. 2541-2543 พบมี
กวาวเครือขาวเจริญอยู่ในป่าเบญจพรรณร่วมกับป่าไผ่ในพื้นที่ลาดเอียง และภูเขา กวาวเครือขาวเจริญ
ได้ทั้งในดินร่วน ดินปนทรายและปนหินกรวด บริเวณที่พบมากที่สุดได้แก่จังหวัดเชียงใหม่และ
กาญจนบุรีลักษณะที่ต่างกันอย่างชัดเจนระหว่างกวาวเครือขาวที่พบในต่างพื้นที่คือดอกและเมล็ด
ดอกกวาวเครือขาวจากพื้นที่ทางภาคเหนือมีสีม่วงเข้มมากกว่าที่พบในภาคกลาง เก็บรวบรวมราก
สะสมอาหารและวิเคราะห์ค่าทางเคมีด้วยวิธีรังคเลขผิบบาง (Thin Layer Chromatography: TLC) พบ
ความแตกต่างในเชิงคุณภาพและปริมาณของพิวรารินจากการวิเคราะห์ด้วยTCL-densitometry
ลักษณะภายนอกเมล็ดมีความแตกต่างกันซึ่งจำแนกตามสี ปลายและรูปร่าง เก็บเมล็ดจากจังหวัด
เชียงใหม่และกาญจนบุรีเพื่อศึกษาความแตกต่างระหว่างพันธุ์ในแปลงปลูก พบว่าไม่มีความแตกต่าง
ระหว่างการเจริญเติบโต การผลิตรากสะสมอาหาร นำรากสะสมอาหารมาวิเคราะห์หาปริมาณ
เปอร์เซ็นต์ของแป้ง โปรตีน ไขมัน กาก ถ้าไม่พบความแตกต่างระหว่างเปอร์เซ็นต์โปรตีน ไขมัน
กาก และถ้า แต่เปอร์เซ็นต์แป้งในสายพันธุ์จากกาญจนบุรีสูงกว่าสายพันธุ์จากเชียงใหม่อย่างมีนัย
สำคัญ ซึ่งยืนยันความแตกต่างระหว่างสายพันธุ์ได้ในเชิงคุณภาพและปริมาณโดยการใช้พิวรารินเป็น
สารมาตรฐานด้วย TLC และ TCL-densitometry

ภาควิชา.....

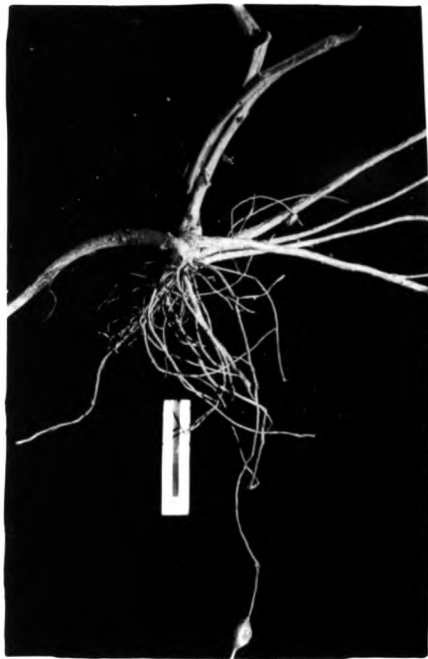
ลายมือชื่อนิสิต..... รัตนา ปานเรียนแสน

สาขาวิชา..... เทคโนโลยีชีวภาพ

ลายมือชื่ออาจารย์ที่ปรึกษา..... 

ปีการศึกษา..... 2543

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....



(A)



(B)

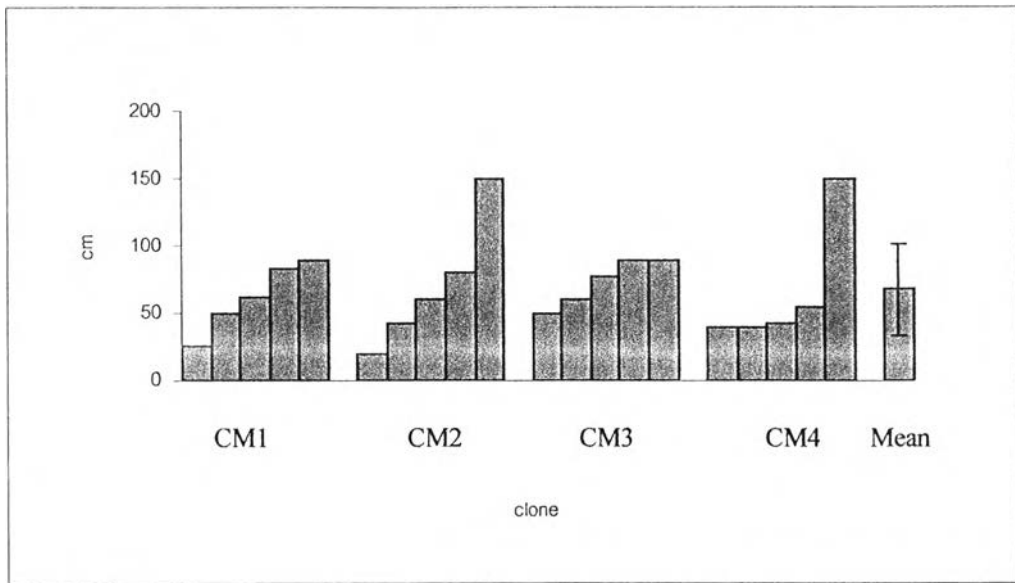


(C)

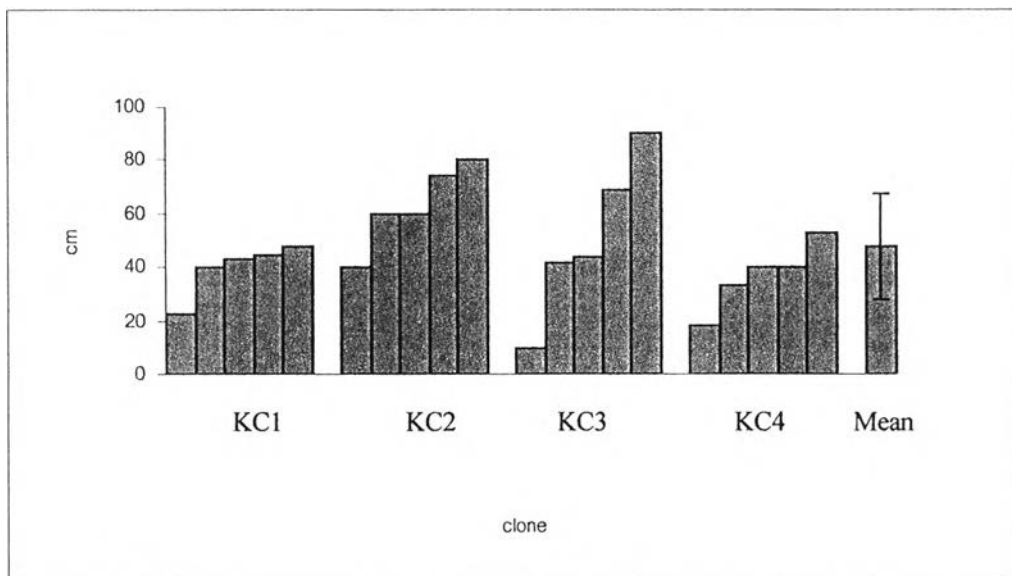


(D)

Figure 18 The field grown *P. mirifica* tuberous root from the Chiangmai and Kanchanaburi variety: (A) no tuberous root, (B) a few tuberous roots (C) numerous tuberous roots and (D) shapes of tuberous roots



(A)



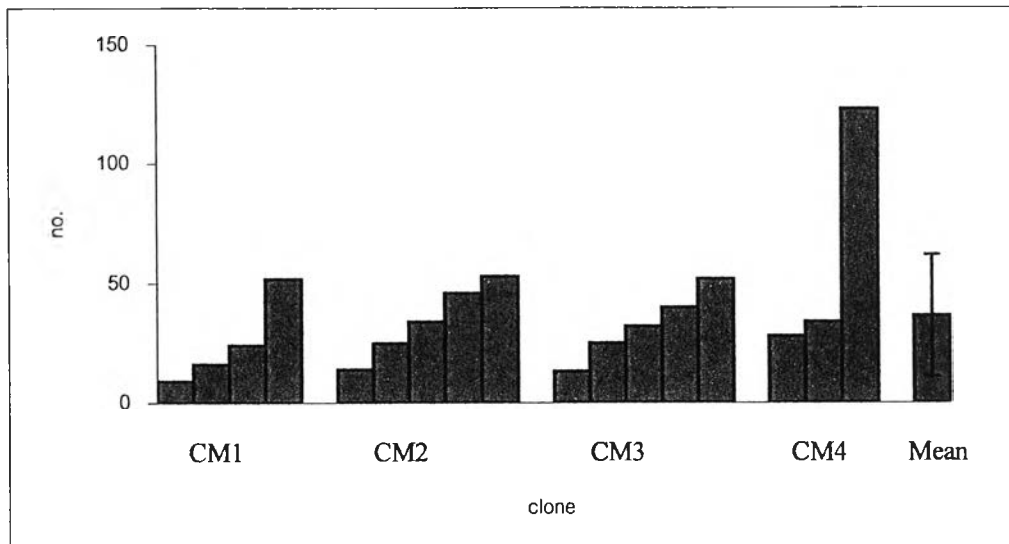
(B)

Figure 19 The main root lengths of the field grown *P. mirifica* from (A) Chiangmai and (B) Kanchanaburi varieties

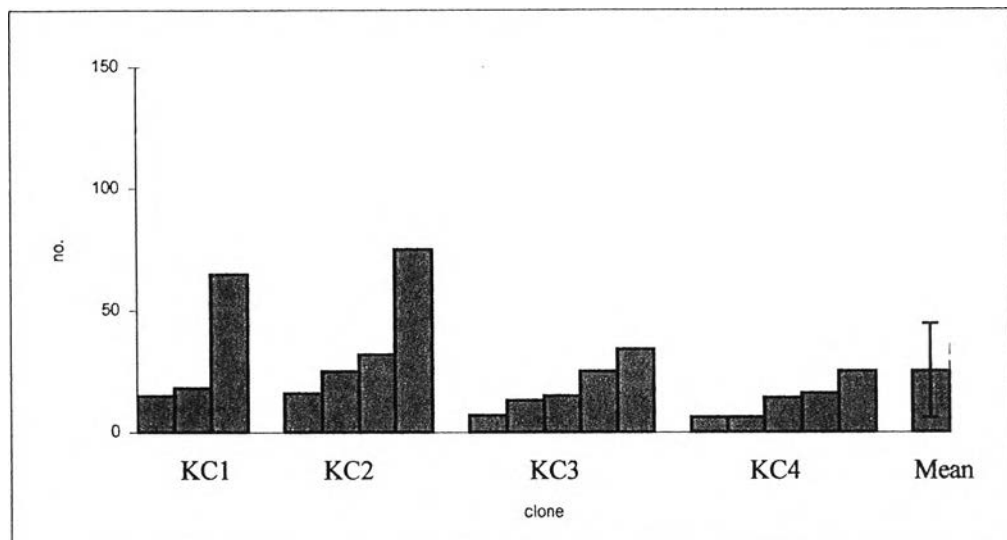
The numbers and weights of tuberous roots of field grown plants are shown in **Table 12, Figure 20 and 22**. The mean and SE values of the tuberous root number of the Chiangmai variety was 36.47 ± 25.55 while that of the Kanchanaburi variety was 25.12 ± 19.33 . Comparison of the tuberous root number between varieties was found to be no difference.

Table 12 The tuberous root number of the field grown *P. mirifica* plant from the Chiangmai and Kanchanaburi varieties

| Variety | No. of tuberous root | | | | | | | |
|--------------|----------------------|-------|-------|-------|-------|-------|-------|-------|
| | CM1 | CM2 | CM3 | CM4 | KC1 | KC2 | KC3 | KC4 |
| Max | 52 | 53 | 52 | 123 | 65 | 75 | 34 | 45 |
| Min | 9 | 14 | 13 | 28 | 15 | 16 | 7 | 6 |
| Mean | 25.25 | 34.4 | 32.4 | 61.67 | 32.7 | 31.6 | 18.8 | 17.4 |
| SD | 18.86 | 15.69 | 14.77 | 53.20 | 28.04 | 26.17 | 10.69 | 16.09 |
| No. of plant | 4 | 5 | 5 | 3 | 3 | 4 | 5 | 5 |



(A)



(B)

Figure 20 The tuberous root number of the field grown *P. mirifica* from (A) the Chiangmai and (B) Kanchanaburi variety

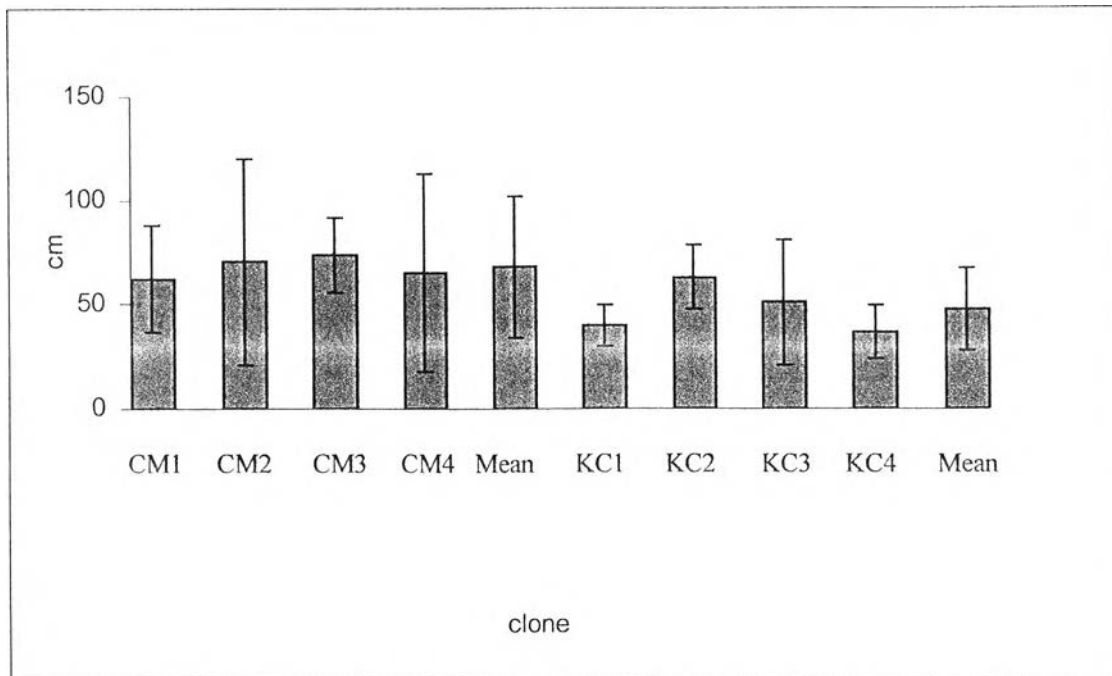


Figure 21 The mean and SD values of the main root length obtained from the field grown *P. mirifica* from the Chiangmai (CM1-4) variety and Kanchanaburi (KC1-4) variety.

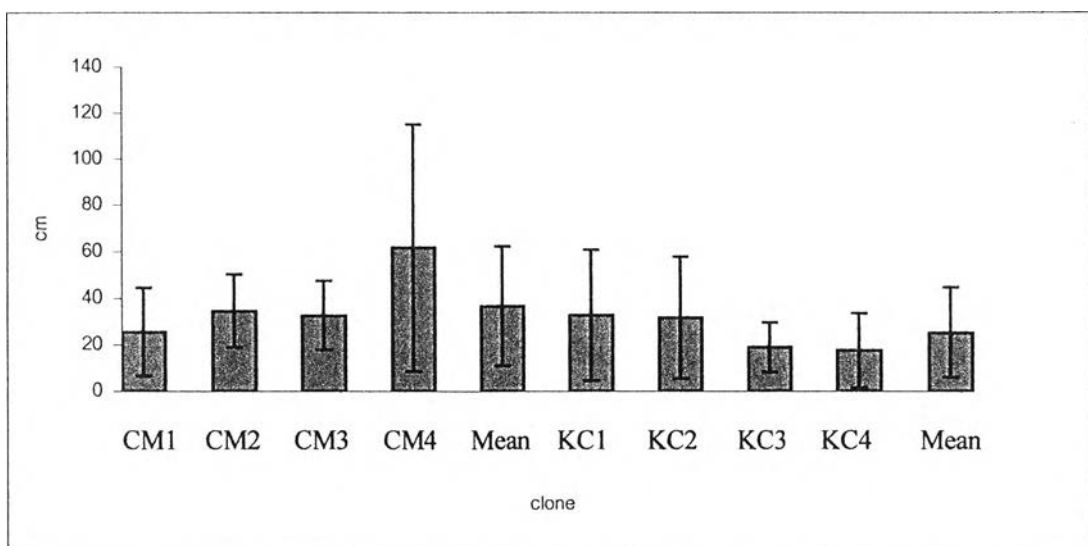


Figure 22 The mean and SD value of the tuberous root numbers obtained from the field grown *P. mirifica* from the Chiangmai (CM1-4) and Kanchanaburi (KC1-4) variety

In the Chiangmai variety, the tuberous root fresh weight was 1.0 - 27.7 kg with the while the mean \pm SE value of 6.86 ± 6.35 kg that of the Kanchanaburi variety was 1.4 - 11.5 kg with the mean \pm SE value of 4.25 ± 2.9 (**Table 13 and Figure 23**). Comparision of the mean value of the fresh weight between varieties was found to be no difference.

The tuberous root dry weight were shown in **Table 14** and **Figure 24**. It was found that the range of dry weight of the Chiangmai and Kanchanaburi variety was 0.13 - 1.25 and 0.11 - 1.10 kg, respectively. The mean \pm SE value of the dry weight of the Chiangmai variety was 0.47 ± 0.27 kg and that of the Kanchanaburi variety was 0.40 ± 0.31 . Comparision of the mean values of the two varieties was found to be no difference.

The percentage of water content in the Chiangmai and Kanchanaburi varieties were 90.95 and 90.22, respectively as shown in **Table 15**

Table 13 The tuberous root fresh weight of the field grown *P. mirifica* in the Chiangmai and Kanchanaburi variety

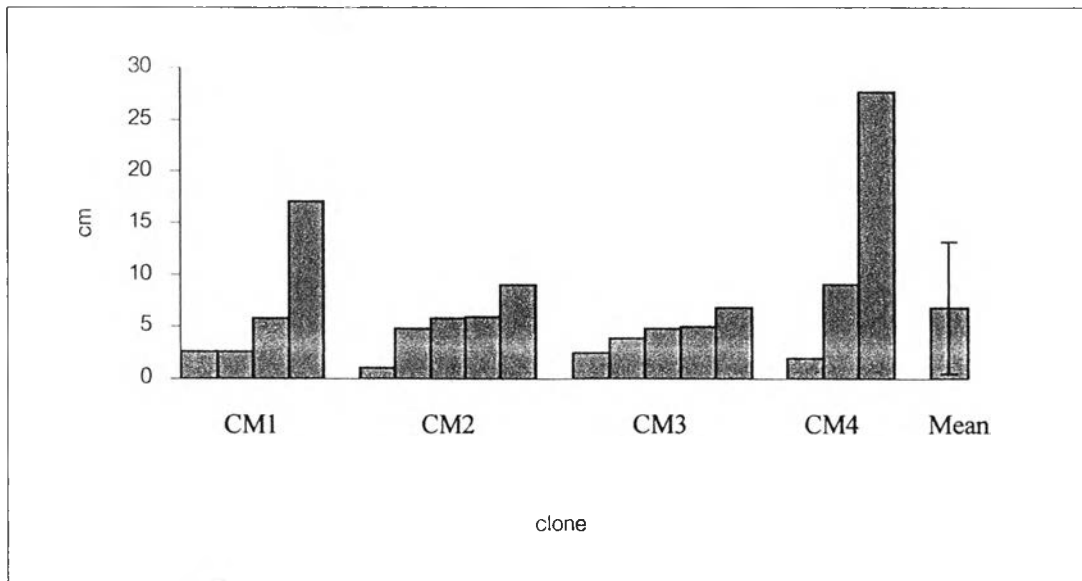
| Variety | Fresh weight (kg) | | | | | | | |
|--------------|-------------------|------|------|-------|------|------|------|------|
| | CM1 | CM2 | CM3 | CM4 | KC1 | KC2 | KC3 | KC4 |
| Max | 17.1 | 9.0 | 6.8 | 27.7 | 8.5 | 11.5 | 4.8 | 4.0 |
| Min | 2.6 | 1.0 | 2.5 | 2.0 | 2.0 | 2.6 | 1.9 | 1.4 |
| Mean | 7.05 | 5.34 | 4.60 | 12.93 | 6.17 | 6.55 | 3.56 | 1.96 |
| SD | 6.88 | 2.88 | 1.58 | 10.84 | 3.62 | 4.11 | 1.41 | 1.14 |
| No. of plant | 4 | 5 | 5 | 3 | 3 | 4 | 5 | 5 |

Table 14 The tuberous root dry weight of the field grown *P. mirifica* plant from in Chiangmai and Kanchanaburi variety

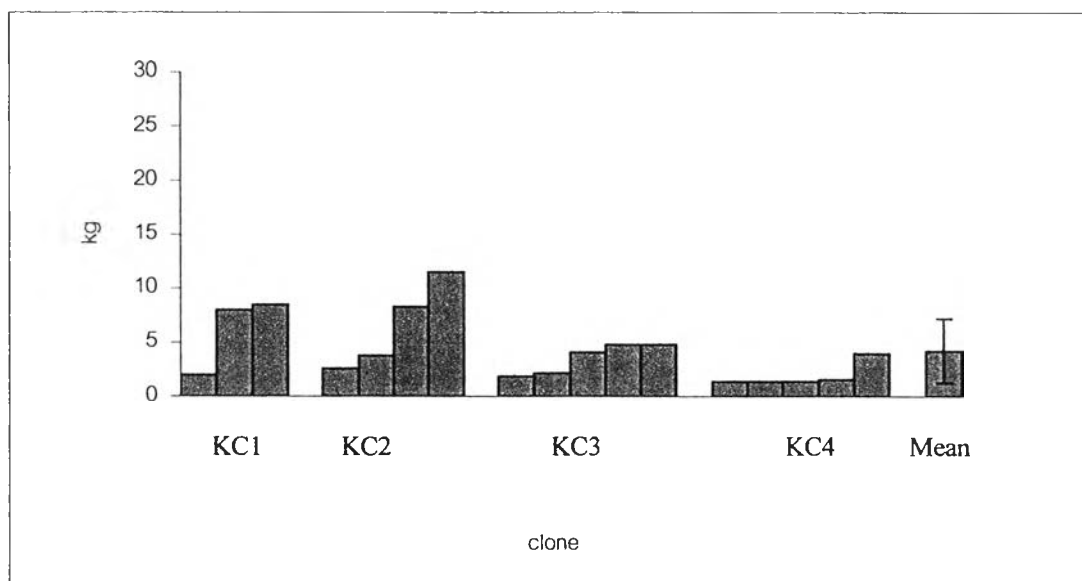
| Variety | Dry weight (kg) | | | | | | | |
|--------------|-----------------|------|------|------|------|------|------|------|
| | CM1 | CM2 | CM3 | CM4 | KC1 | KC2 | KC3 | KC4 |
| Max | 0.68 | 0.56 | 0.58 | 1.25 | 1.05 | 1.10 | 0.74 | 0.35 |
| Min | 0.50 | 0.13 | 0.15 | 0.17 | 0.52 | 0.11 | 0.16 | 0.11 |
| Mean | 0.59 | 0.35 | 0.36 | 0.72 | 0.71 | 0.44 | 0.39 | 0.18 |
| SD | 0.10 | 0.21 | 0.17 | 0.54 | 0.30 | 0.43 | 0.25 | 0.10 |
| No. of plant | 4 | 5 | 5 | 3 | 3 | 4 | 5 | 5 |

Table 15 Water content of the field grown *P. mirifica* tuberous roots obtained from the Chiangmai and Kanchanaburi variety

| No. | Chiangmai variety | | | Kanchanaburi variety | | |
|------|-------------------|-----------------|-----------------|----------------------|-----------------|-----------------|
| | Fresh weight (kg) | Dry weight (kg) | % water content | Fresh weight (kg) | Dry weight (kg) | % water content |
| 1 | 2.60 | 0.50 | 80.77 | 2.00 | 0.52 | 73.80 |
| 2 | 2.60 | 0.68 | 73.85 | 8.00 | 0.55 | 93.13 |
| 3 | 5.90 | 0.50 | 91.47 | 8.50 | 1.05 | 87.61 |
| 4 | 17.10 | 0.68 | 96.02 | 2.60 | 0.11 | 95.81 |
| 5 | 1.00 | 0.13 | 87.40 | 3.80 | 0.25 | 93.37 |
| 6 | 4.80 | 0.15 | 96.94 | 8.30 | 0.32 | 96.11 |
| 7 | 5.90 | 0.41 | 93.02 | 11.50 | 1.10 | 90.48 |
| 8 | 6.00 | 0.53 | 91.25 | 1.90 | 0.16 | 91.58 |
| 9 | 9.00 | 0.56 | 93.80 | 2.20 | 0.18 | 92.05 |
| 10 | 2.50 | 0.15 | 94.16 | 4.10 | 0.32 | 92.10 |
| 11 | 3.90 | 0.25 | 93.51 | 4.80 | 0.57 | 88.23 |
| 12 | 4.80 | 0.33 | 93.04 | 4.80 | 0.74 | 84.69 |
| 13 | 5.00 | 0.47 | 90.54 | 1.40 | 0.11 | 92.14 |
| 14 | 6.80 | 0.58 | 91.49 | 1.40 | 0.13 | 90.71 |
| 15 | 2.00 | 0.17 | 91.40 | 1.40 | 0.14 | 89.93 |
| 16 | 9.10 | 0.73 | 91.95 | 1.60 | 0.15 | 90.69 |
| 17 | 27.70 | 1.25 | 95.49 | 4.00 | 0.35 | 91.35 |
| Mean | 6.86 | 0.47 | 90.95 | 4.25 | 0.40 | 90.22 |
| SE | 6.35 | 0.27 | 5.57 | 2.98 | 0.31 | 4.91 |

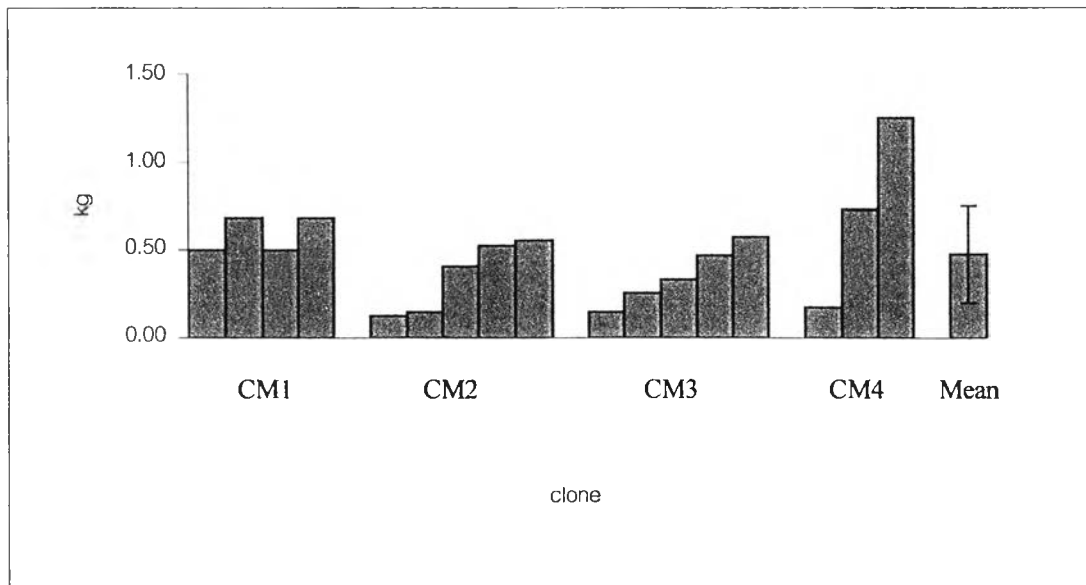


(A)

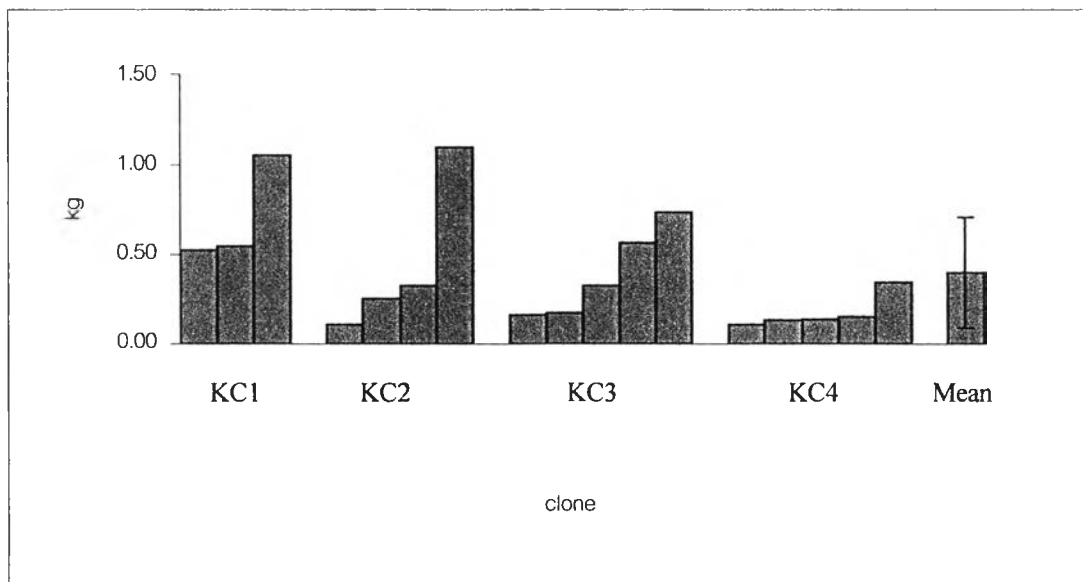


(B)

Figure 23 The tuberous root fresh weight of the field grown *P. mirifica* from (A) the Chiangmai and (B) Kanchanaburi variety



(A)



(B)

Figure 24 The tuberous root dry weight of the field grown *P. mirifica* from (A) the Chiangmai and (B) Kanchanaburi variety

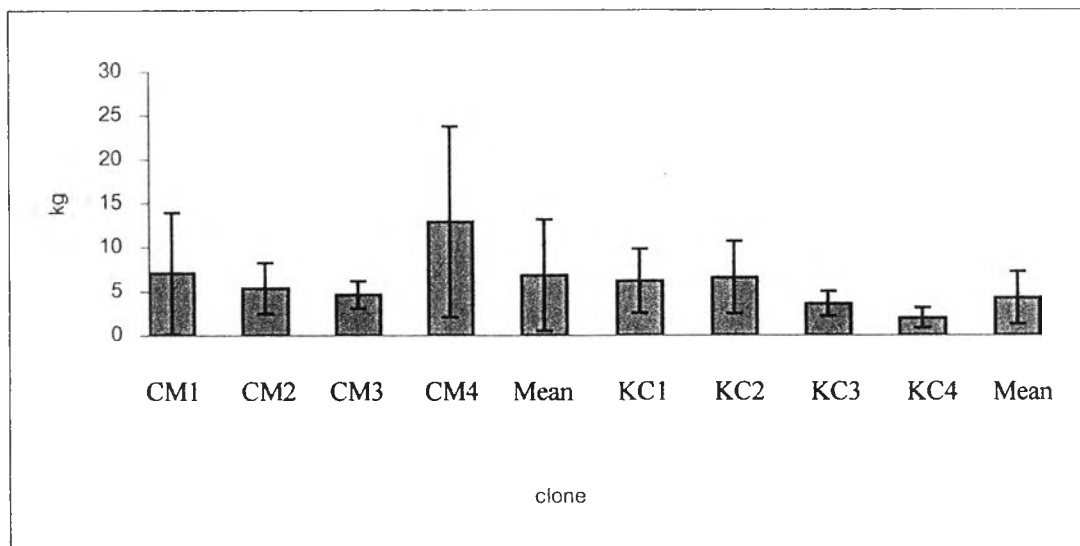


Figure 25 The mean and SD values of the tuberous root fresh weights of the field grown *P. mirifica* from the Chiangmai (CM1-4) and Kanchanaburi (KC1-4) variety

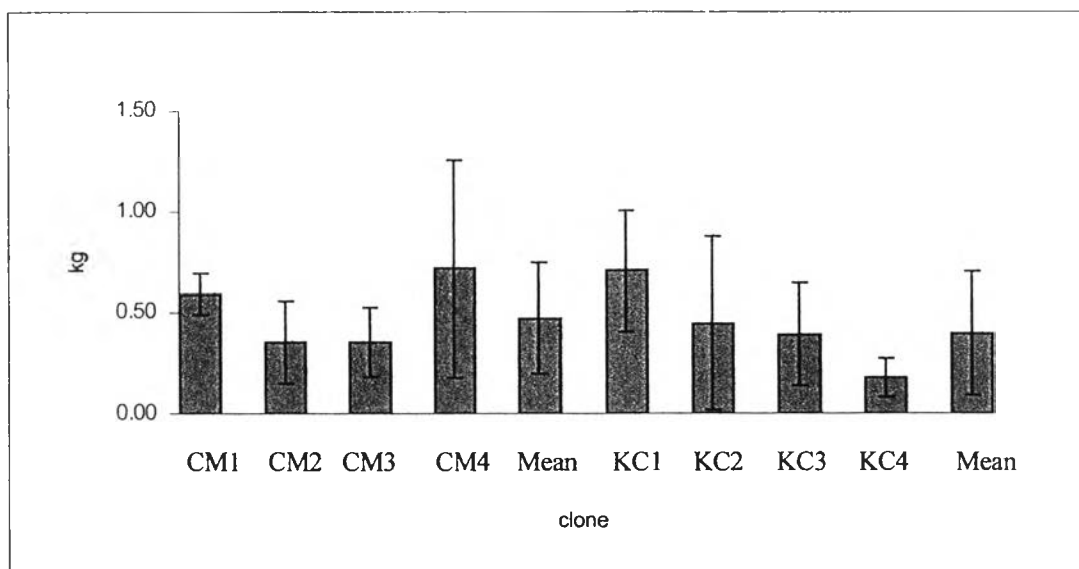


Figure 26 The mean and SD values of the tuberous root dry weight of the field grown *P. mirifica* from the Chiangmai (CM1-4) and Kanchanaburi (KC1-4) variety

2.3 The proximate analysis of *P. mirifica* tuberous roots from field grown plants

Table 16 shows the the proximate analysis result of field grown plants of the Chiangmai and Kanchanaburi variety. It was found that the mean \pm SE value of starch in the Kanchanaburi variety was significantly higher ($p < 0.05$) than the Chiangmai variety. The mean values of the protein, fat, fiber and ash were not statistically difference.

Table 16 Proximate analysis of *P. mirifica* tuberous roots (dry weight basis)

| Variety | % starch | % protein | % fat | % fiber | % ash |
|---------------|------------------|------------------|-----------------|------------------|------------------|
| CM1 | 14.95 | 11.66 | 0.44 | 18.80 | 12.96 |
| CM2 | 18.89 | 3.86 | 0.50 | 14.28 | 13.11 |
| CM3 | 15.93 | 10.15 | 0.30 | 16.66 | 12.02 |
| CM4 | 16.51 | 5.62 | 0.32 | 14.73 | 13.75 |
| Mean \pm SD | 16.57 \pm 1.80 | 7.82 \pm 3.68 | 0.39 \pm 0.10 | 16.12 \pm 2.07 | 12.96 \pm 0.71 |
| KC1 | 16.63 | 16.63 | 0.44 | 14.60 | 14.91 |
| KC2 | 21.47 | 10.4 | 0.42 | 15.60 | 13.68 |
| KC3 | 36.34 | 6.00 | 0.32 | 10.50 | 8.54 |
| KC4 | 25.01 | 7.33 | 0.22 | 14.01 | 12.43 |
| Mean \pm SD | 24.86 \pm 8.48 | 10.09 \pm 4.73 | 0.35 \pm 0.10 | 13.68 \pm 2.22 | 12.39 \pm 2.76 |

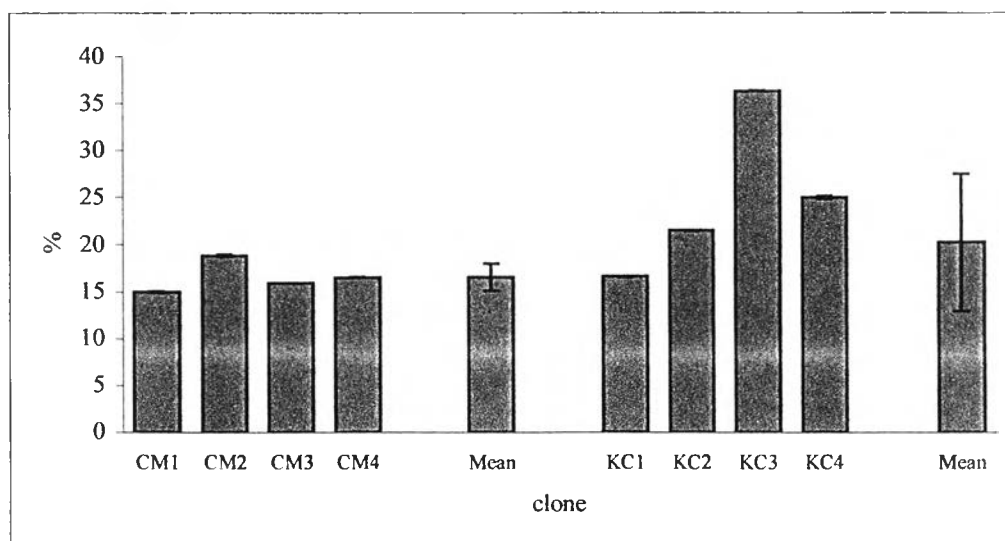


Figure 27 The percentage of the starch content of field grown *P. mirifica* tuberos roots from (A) the Chiangmai and (B) Kanchanaburi variety

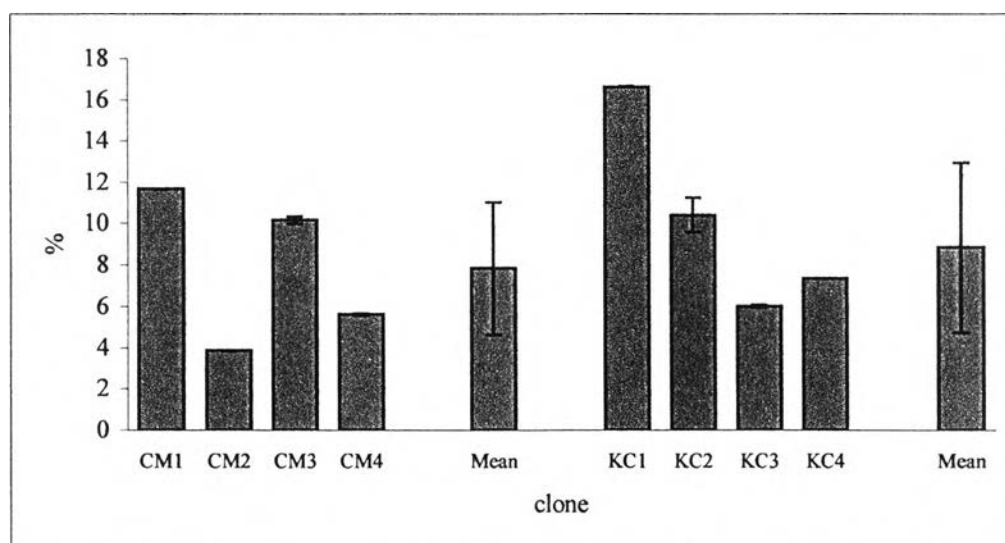


Figure 28 The percentages of protein content of field grown *P. mirifica* from tuberos roots (A) the Chiangmai and (B) Kanchanaburi variety

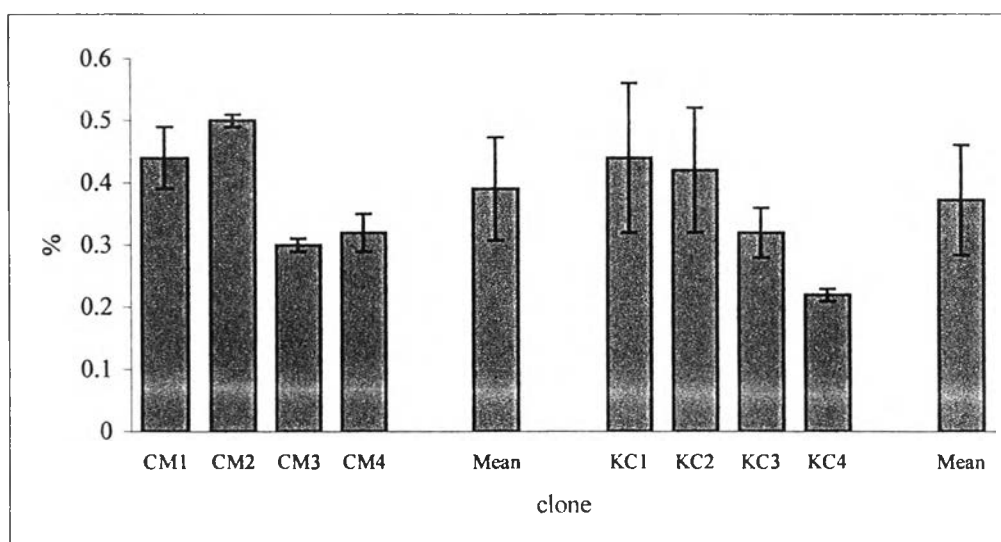


Figure 29 The percentage of the fiber content of field grown *P. mirifica* tuberos roots from (A) the Chiangmai and (B) Kanchanaburi variety

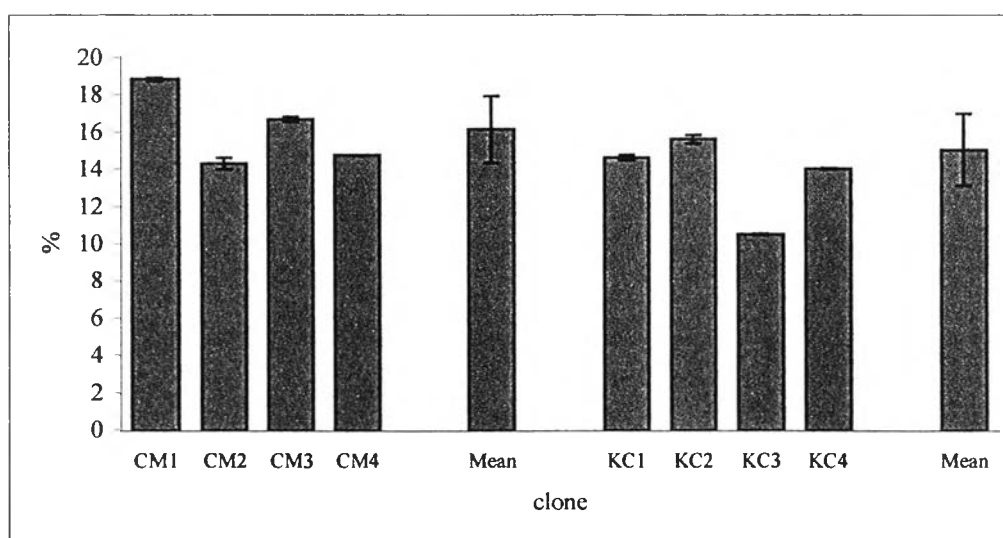


Figure 30 The percentages of the fat content of field grown *P. mirifica* tuberos roots from (A) the Chiangmai and (B) Kanchanaburi variety

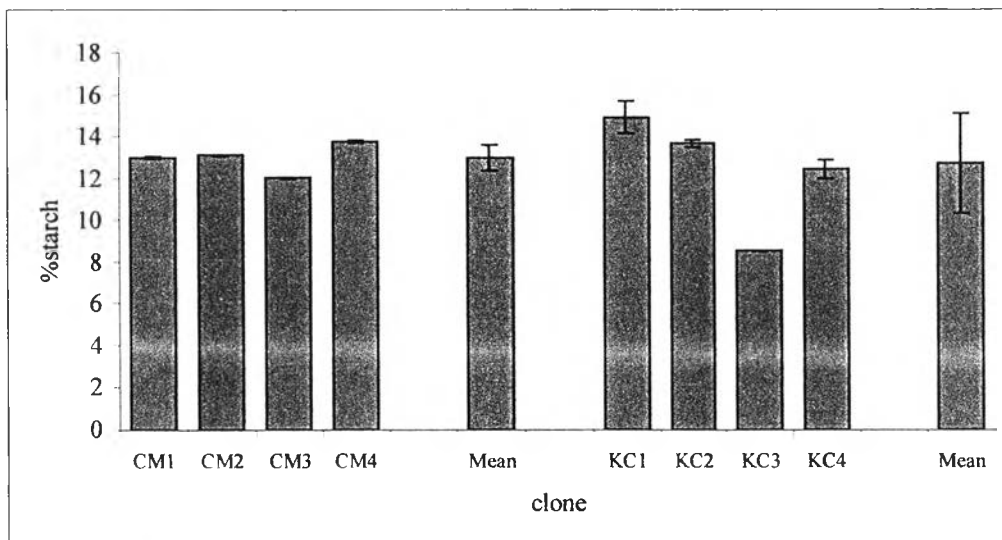
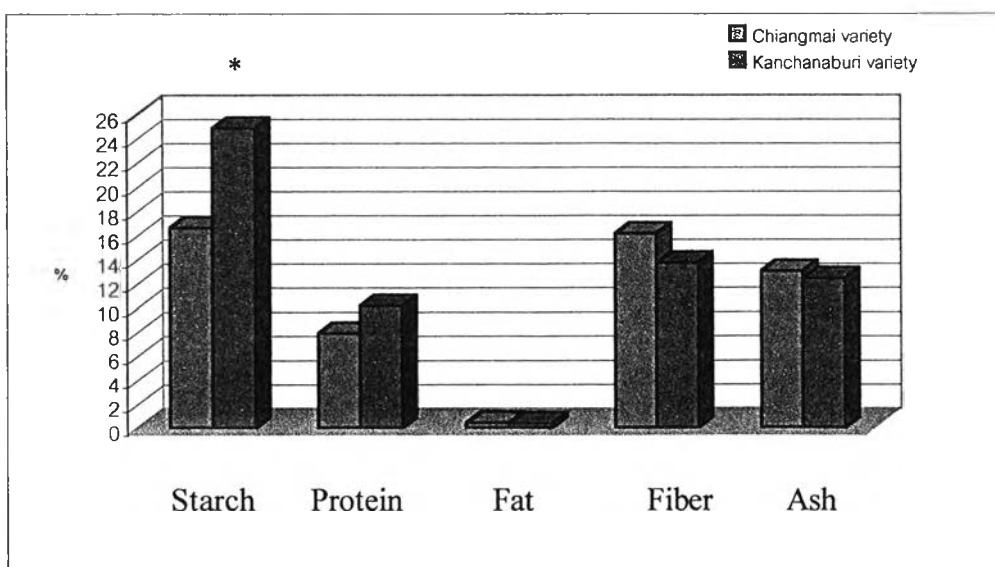


Figure 31 The percentage of the ash content of field grown *P. mirifica* tuberos roots from (A) the Chiangmai and (B) Kanchanaburi variety



* showed the significant different between variety at $p < 0.05$

Figure 32 The mean values of the percent starch, protein, fat, fiber and ash obtained from the field grown *P. mirifica* tuberos roots from the Chiangmai and Kanchanaburi variety

2.4 Quantitative analysis of puerarin in field grown *P. mirifica* tuberous roots by TLC-densitometry

2.4.1 Setting up the separation method for puerarin using TLC method

The composition of chloroform-methanol-H₂O with the ratio of 40:40:2 was found to be the optimum solvent system for the separation of puerarin. Under these conditions, a blue band is clearly demonstrated (**Figure 33**). The R_f value was found to be approximately 0.70 .

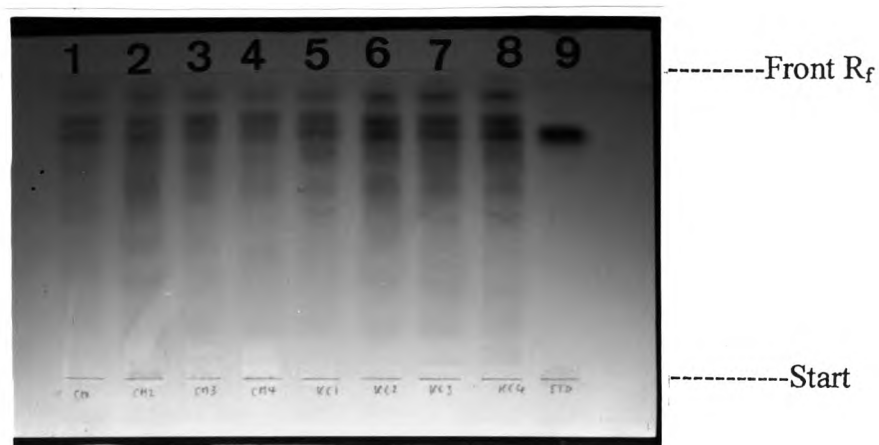
2.4.2 The quantitative analysis of the extracted puerarin from field grown *P. mirifica*

Based on the TLC densitometry, all the tuberous roots from the Chiangmai and Kanchanaburi variety contained variable amounts of puerarin (**Table 17**). The Chiangmai variety contained 0.0724 – 2.8416 %w/w with the mean \pm SE value of 0.9651 ± 0.7048 while the Kanchanaburi variety contained 0.3922 – 2.3581 %w/w with the mean \pm SE of 1.2892 ± 0.6328 %w/w. The Kanchanaburi variety appeared to contain significant ($P < 0.05$) higher puerarin content than that of the Chiangmai variety. In addition, the mean puerarin content in all clones in the Kanchanaburi variety was higher than that of the Chiangmai variety, whereas there was no significant difference among the clones.

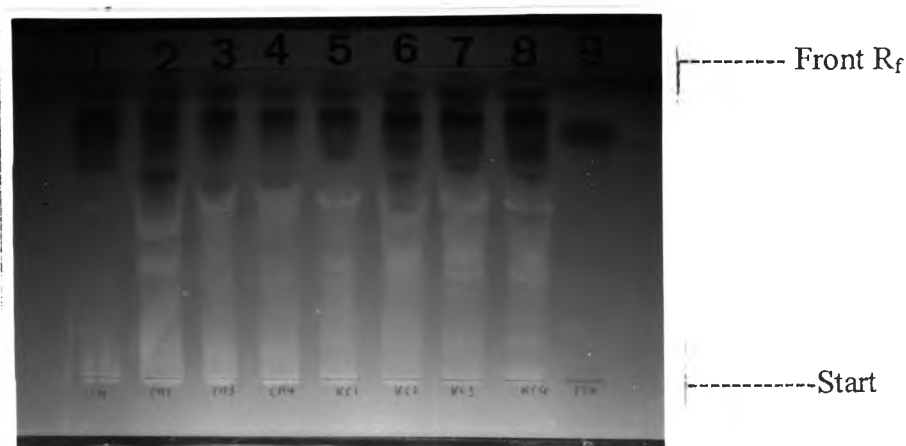
Table 17 The percentages of puerarin contents in *P. mirifica* tuberous roots obtained from TLC densitometric method

| Variety | % dry weight of puearin content | | | | | | | |
|--------------|---------------------------------|--------|--------|--------|--------|--------|--------|--------|
| | CM1 | CM2 | CM3 | CM4 | KC1 | KC2 | KC3 | KC4 |
| Max. | 2.1802 | 2.8416 | 1.3377 | 0.6443 | 2.2849 | 2.3581 | 2.0394 | 0.6497 |
| Min. | 0.0938 | 0.0724 | 0.3693 | 0.0895 | 0.4169 | 1.4945 | 0.3922 | 0.4766 |
| Mean | 0.8583 | 1.1614 | 1.0825 | 0.4423 | 1.3547 | 1.9557 | 1.1326 | 0.5534 |
| SD | 0.8300 | 1.0630 | 0.4085 | 0.3066 | 0.9340 | 0.4432 | 0.5914 | 0.0882 |
| No. of plant | 4 | 5 | 5 | 3 | 4 | 5 | 5 | 3 |

CM = Chiangmai variety, KC = Kanchaburi variety

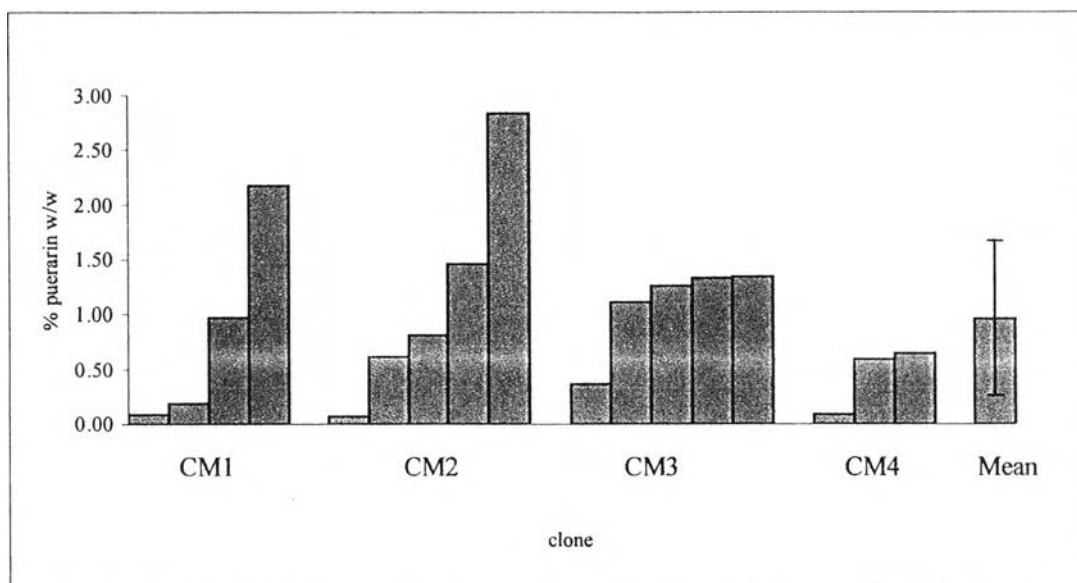


(A)

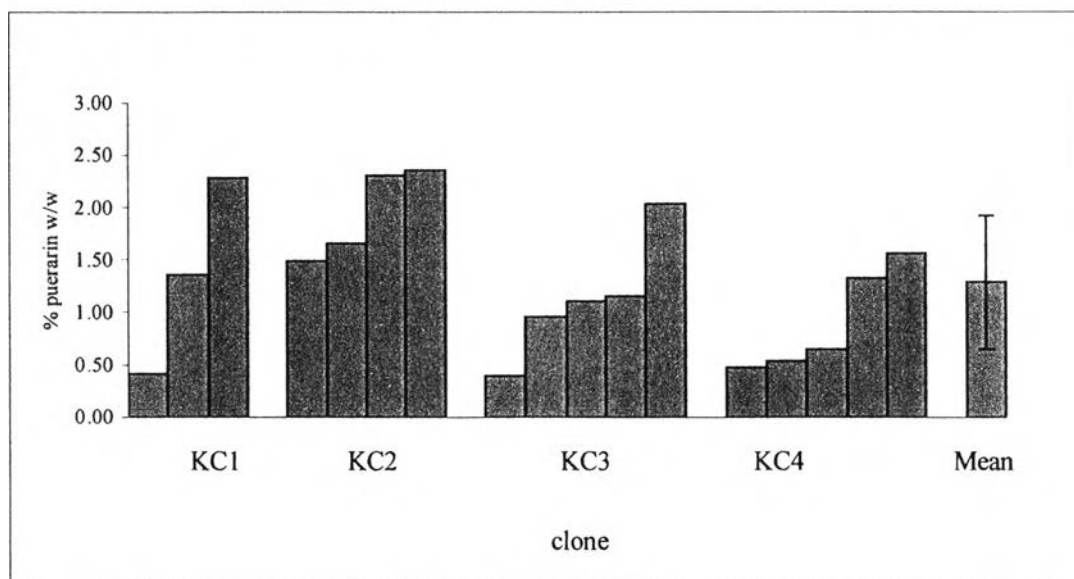


(B)

Figure 33 TLC patterns of the extracted puerarin using solvent system: chloroform : methanol : H₂O at ration 40 : 40 : 2. TLC patterns were obtained from two varieties of *P. mirifica* tuberous root and the standard puerarin (1-4) the Chiangmai variety (5-8) the Kanchanaburi variety (9) Standard puerarin under UV- visible light at (A) 254 and (B) 365 nm



(A)



(B)

Figure 34 Puerarin contents in *P. mirifica* tuberos roots from (A) the Chiangmai and (B) Kanchanaburi variety

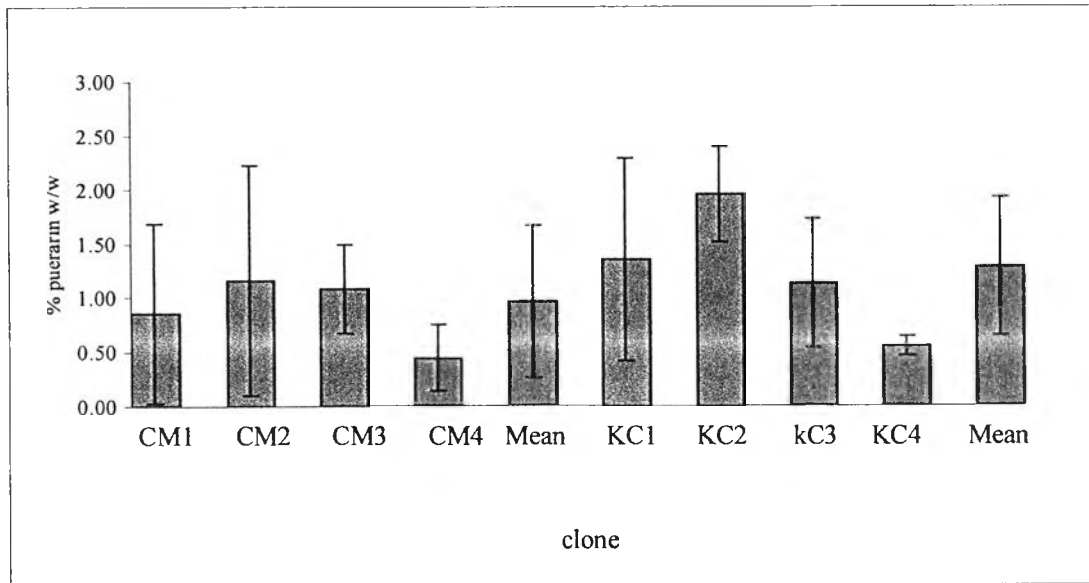


Figure 35 Mean and SD value of puerarin content in *P. mirifica* tuberous roots from (A) the Chiangmai and (B) Kanchanaburi variety

Table 18 Summariz of the yield of the field grown *P. mirifica* from the Chaingmai and Kanchanburi variety

| Yield | Chiangmai | Kanchanaburi |
|-------------------------|----------------|---------------|
| Peristem length (cm) | 10.15 ± 3.32 | 9.45 ± 3.41 |
| Number of tuberous root | 34.06 ± 25.12 | 26.46 ± 19.33 |
| Main root length (cm)* | 67.40 ± 32.99 | 47.50 ± 19.69 |
| Fresh weight (kg) | 6.88 ± 6.34.25 | 4.25 ± 2.98 |
| Dry weight (kg) | 0.45 ± 0.27 | 0.41 ± 0.33 |
| % starch* | 16.56 ± 1.80 | 24.86 ± 8.48 |
| % protein | 7.82 ± 3.68 | 10.09 ± 4.73 |
| % fat | 0.39 ± 0.10 | 0.35 ± 0.10 |
| % fiber | 16.12 ± 2.07 | 13.68 ± 2.22 |
| % ash | 12.96 ± 0.71 | 12.39 ± 2.76 |
| % puerarin* | 0.94 ± 0.74 | 1.30 ± 0.67 |

* showed the signifiant different between variety at $p < 0.05$