

SELECTION FOR LOW Δ^9 -TETRAHYDROCANNABINOL CONTENT IN THAI HEMP CULTIVARS

W. KUNKAEW^{1*}, S. JULSRIGIVAL¹, P. TIPPARAT² and S. PINMANEE³

SUMMARY

Selection for reduced Δ^9 -tetrahydrocannabinol (THC) content in four Thai hemp cultivars (including V50, Mae Sa Mai, Huay Hoi and Pang Ung) was carried out in highland areas in the northern Thailand. Research work was conducted for two consecutive growing seasons during 2008 to 2009 at Pangda Royal Agricultural Station, Samoeng district, Chiang Mai province, Thailand. Results of selection indicated that after selecting for two successive generations, the average THC content of four Thai hemp cultivars reduced to 18.0-55.0% and cannabidiol (CBD) content increased to 20.0-127.0%. The results of selection also indicated that chemotype classification could be grouped by using the ratio of CBD/THC content as follows: non-drug type (CBD/THC>10.0), intermediate type ($1.0 \leq \text{CBD/THC} \leq 10.0$) and drug type (CBD/THC<1.0). Thus, selection for reduced THC content, high ratio of CBD/THC content could be used as alternative criteria for improving low THC content in hemp cultivars. As well, mass selection method was considered as an effective and suitable method for improving these THC and CBD traits.

Key words: chemotype, hemp, THC, CBD

INTRODUCTION

Hemp (*Cannabis sativa* L.) has been a source of fiber crops for a long time. Hemp has been cultivated by Hmong, the hill tribe people who live in the highland areas in northern Thailand. In Thailand, hemp is classified as a narcotic crop which farmers are prohibited to grow. However, this crop is still planted in some outreach areas where it cannot be noticed by government officers. Her Majesty the Queen is very much interested in hemp crop cultivation for fiber products and kindly recommended to the relevant government sectors to promote hemp cultivation in the highland areas since 2004.

1 Royal Project Foundation, 65 Suthep Road, Chiang Mai 50200, Thailand.

2 Regional Medical Sciences Center, 191 M. 8, T. Don Kaew, A. Mae Rim, Chiang Mai 50180, Thailand.

3 Highland Research and Development Institute (Public Organization), 65 Suthep Road, Chiang Mai 50200, Thailand.

* Corresponding author: weeraupun@hotmail.com

Research work on cultivation and development of hemp in Thailand were first studied and reported by Queen Sirikit Botanic Garden in Chiang Mai, Thailand. Later, the Economic and Social Institute of Thailand granted financial funds to the Royal Project Foundation and to the Highland Research and Development Institute (Public Organization) to conduct research works on hemp production and varietal improvement for low Δ^9 -tetrahydrocannabinol (THC) content.

In some European countries, monoecious fiber hemp is considered as an economic crop which can be grown legally by farmers if THC content is lower than 0.2 percent (Mechtler *et al.*, 2004; Callaway, 2008). Presently, it is found that demand of hemp products which is produced widely for industrial uses such as fibers, seeds, oils, and medicines are increasing (Ranalli, 1999; Johnson, 2010). Thus, hemp is widely and commonly planted for fiber and/or seed production in many countries in Europe and some countries in Asia where climatic and soil factors are suitable for planting.

In Australia, studies on adaptation of hemp varieties indicated that varieties introduced from sub-tropical climates were able to adapt in Australia better than varieties brought from temperate areas. In addition, analysis of THC content of these introduced varieties showed evidently higher values than their places of introduction. Some varieties gave THC content as high as 1.0%. Hot weather which is a climatic factor to inhibit plant growth and development as well as increasing of THC content was reported by Jobling and Warner (2001). Furthermore, it is also found that local hemp varieties possess THC content more than 0.5% which is prohibited to be grown by farmers. Therefore a varietal improvement program for decreasing THC content in hemp was first initiated in Thailand by Kunkaew *et al.* (2009, 2010). Their studies indicated that THC content was different among varieties and varied quantitatively within populations of each variety. The variation of THC content could be classified into three chemotype groups, including drug, intermediate and non-drug type groups. Kaveeta *et al.* (2006) and Sengloung *et al.* (2009) studied the morphology, growth and development of hemp crop growing on the highland areas in Thailand.

Chemical components in hemp plant were studied and reported by many researchers. An important chemotype of hemp is cannabinoid which is one of the terpenophenolic compounds and is a unique character to cannabis plants. These terpenophenolic compounds are produced by glandular trichomes that occur on most aerial surfaces of the plants. Two important compounds of cannabinoid are Δ^9 -tetrahydrocannabinol content or THC and cannabidiol or CBD. Since THC is classified as one kind of narcotic substances, thus, the amount of THC content in plant is an important data for dividing hemp plant into drug and non-drug type. Thus, information on THC and CBD content in hemp are very useful in guiding low THC content improvement in hemp variety (Hillig and Mahlberg, 2004). Genetic control for THC and CBD content in hemp have been previously studied and reported. De Meijer *et al.* (2003) and Mandolino *et al.* (2003) found that THC and CBD traits were controlled by a single locus gene (B) with two co-dominant alleles (B_T and B_D). Therefore, pure CBD plant or fiber type has a B_D/B_D , while pure THC plant or drug type has a B_T/B_T genotype at B locus and intermediate type has the heterozygous of B_D/B_T genotype which two different alleles B_D and B_T having as co-dominant control. The ratio of CBD/THC indicates the qualitative character of these

chemotypes. It was found that hybrid F1 derived from crossing between pure CBD and THC plants would give all heterozygous (CBD/THC) plants. The segregation of the CBD, CBD/THC and THC chemotypes was observed in accordance with a 1:2:1 ratio, which suggests a single Mendelian locus determining chemotype, with two co-dominant alleles, one for CBD and another for THC. It was further investigated that B_D/B_D genotype had a lethal effect for seed fertility and viability. B_T/B_T genotype had more vigor for these two traits than B_D/B_D genotype. These inferior problems of B_D/B_D genotype may probably be due to semi-lethal effect which is caused by co-dominant pairing of two alleles (de Meijer *et al.*, 2003).

Hennink (1994) studied the inheritance of THC and CBD traits in hemp and reported that narrow-sense heritability (h^2_n) of THC and CBD traits was rather low, estimated about 15.0% and 8.0%, respectively, in contrast with results of Kunkaew *et al.* (2010) who reported that heritability of THC trait of local hemp cultivars estimated under highland growing condition in Thailand was about 71-87%. Population improvement in crop plant could be made by simple mass selection method, especially improving of qualitative traits which are controlled by a few genes (Allard, 1960). De Meijer and van Soest (1992) and Hennink (1994) were successful in developing low THC content varieties in hemp crops by using mass selection method.

The objectives of this research work are to develop low THC content in local hemp varieties for growing as an economic fiber and oil seed crop on the highland areas in the northern part of Thailand.

MATERIALS AND METHODS

In 2008 growing season (during June – December), original or first generation population (M0) of four local hemp cultivars collected in Thailand which included V50, Mae Sa Mai, Huay Hoi and Pang Ung were prepared for THC and CBD identification. The experiment was conducted in plastic shelter growing condition at Pangda Royal Agricultural station (elevation 720 m above mean sea level), Samoeng District, Chiang Mai Province, Thailand. The average temperature at this station was 18.8 – 29.6 °C (mean was 23.4 °C), average air humidity was 53.6 – 95% (mean was 74.3%) and rainfall was 1,075.5 mm. About 80 days and 95-120 days after sowing which were early stages of flowering of male and female plants, respectively, 50 male plants and 100 female plants were randomly sampled. Leaves on the top parts of stem (about 1/3 of stem) of both male and female plants were collected separately for THC and CBD content analysis. Gas chromatography method was used for analyzing these two chemotypes content (United Nations, 1987). The analysis was conducted at the laboratory of Regional Medical Sciences Center in Chiang Mai Province. After the analysis results were obtained, THC content data of both male and female plants of each cultivar were examined for plant which its THC content was lower than 0.3% and then individual plant was selected. These low THC content plants of both sexes were still kept in the plots for producing advanced generation hybrid seeds while the undesirable plants were rogued. At maturity, female plants were harvested and seeds were bulked together in order to develop first selection cycle (M1) generation.

In 2009 growing season, seeds of M1 generation were planted and collection of leaves in both male and female plants of each hemp cultivar, THC and CBD analysis were carried out the same as M0 generation.

THC and CBD data of each hemp cultivar of both M0 and M1 generations were classified into three chemotype groups which included (1) non-drug type (THC<0.3%), (2) intermediate type (THC>0.3% and CBD>0.5%) and drug type (THC>0.3% and CBD<0.5%) which was proposed by de Meijer *et al.* (1992). Relationship between THC and CBD traits for distinguishing among the chemotype groups was examined by simple regression method (Steel and Torrie, 1960).

RESULTS

Analysis of chemotype contents and classifications of 4 hemp cultivars are presented in Tables 1-4 and Figures 1-4. Results of each cultivar are described as follow:

1. V50 cultivar.

A. Chemotype composition:

(a) THC content. Analysis of chemotype content of this hemp cultivar showed that average THC content obtained from male and female plants of original population (M0-generation) were 0.62% and 0.40%, respectively, average of both sexes was 0.47%. After one generation of selection (M1-generation), average THC content of male and female plants decreased to 0.24% and 0.20%, respectively. Average of both sexes decreased to 0.21% or decreased about 55.0% from M0 generation.

(b) CBD content. It was found that average CBD content of M0 generation for male and female plants were 1.01% and 0.72%, respectively, average of both sexes was 0.82%. In M1-generation, CBD content of male plants reduced to 0.85% but female plants increased to 1.04%, averaged of both sexes increased to 0.98% or increased from M0 generation about 20.0%.

B. Chemotype classification:

(a) Non-drug type. It was found that non-drug type of M0 generation consisted of 42.0% and 48.0% of male and female plants, respectively, average of both sexes was 45.0%. In M1 generation, average of male and female plants increased to 60.5% and 74.7%, respectively, average of both sexes increased to 67.5% or increased about 50.0% from M0 generation. For association analysis between THC and CBD plant of non-drug type, it indicated that there was high positive significant relationship between THC and CBD content of this chemotype group with $R^2=0.86^{**}$.

(b) Intermediate type. It was found that M0 generation consisted of 26.0% of male and 32.0% of female plants, averaged 29.0% of both sexes. In M1 generation, average of male and female plants increased to 34.9% and 23.2%, respectively, average of both sexes increased slightly to 29.1%. There was high positive significant relationship between THC and CBD content of this chemotype group with $R^2=0.91^{**}$.

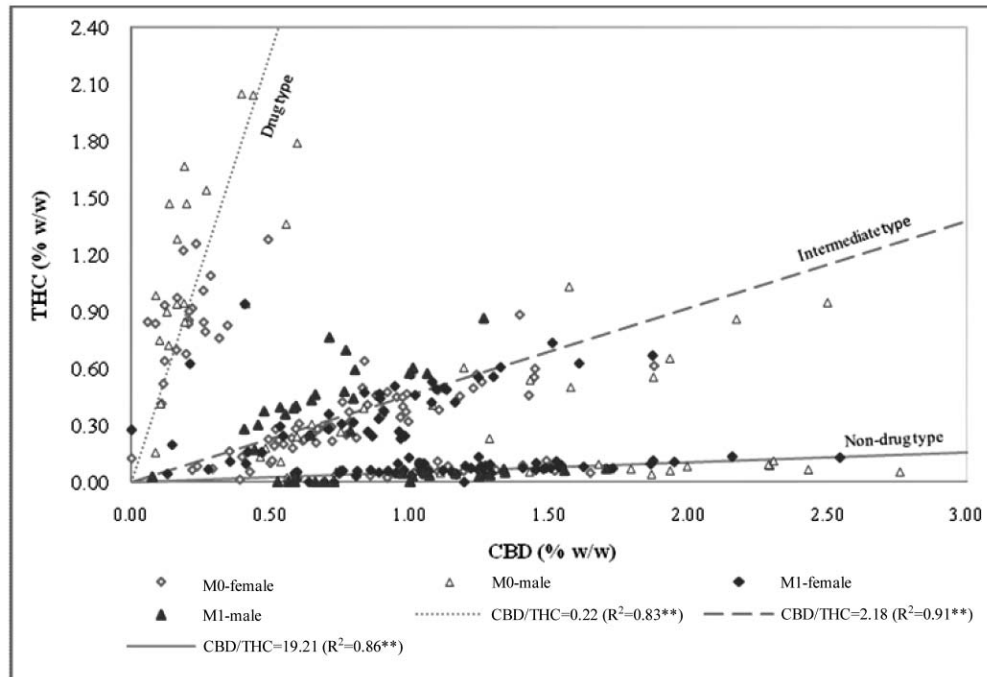


Figure 1. THC and CBD contents of individual plant belongs to M0 and M1 of V50 hemp cultivar and relationship between THC and CBD of each chemotype group. The experiment was conducted at Pangda Agricultural Station in 2008 and 2009 growing seasons.

Table 1. THC content, CBD content and chemotype group of M0 and M1 of V50 hemp cultivar.

Chemotype content/group	M0			M1		
	Male	Female	Average	Male	Female	Average*
A) Chemotype content						
a) THC content (%)	0.62	0.40	0.47	0.24	0.20	0.21 (-55%)
b) CBD content (%)	1.01	0.72	0.82	0.85	1.04	0.98 (+20%)
B) Chemotype group						
a) Non-drug type (%)	42.0	48.0	45.0	60.5	74.7	67.5 (+50%)
b) Intermediate type (%)	26.0	32.0	29.0	34.9	23.2	29.1 (+0%)
c) Drug type (%)	32.0	20.0	26.0	4.6	2.1	3.4 (-87%)

* Values in brackets are percentages of increasing or decreasing chemotype content and chemotype groups.

(c) Drug type. It was found that M0 generation consisted of 32.0% and 20.0% of male and female plants, respectively, averaged 26.0% of both sexes. In M1 generation, average of male and female plants reduced to 4.6% and 2.1%, respectively, average of both sexes decreased slightly to 3.4% or decreased about 87.0% from M0 generation. There was a high positive significant relationship between THC and CBD content for this chemotype group with $R^2=0.83^{**}$.

2. Mae Sa Mai cultivar.

A. Chemotype composition:

(a) THC content. The average THC contents of male and female plants of M0 generation were 0.79% and 0.62%, respectively, and the average of both sexes was 0.68%. In M1 generation, average THC content of male and female plants decreased to 0.45% and 0.38%, respectively, average of both sexes decreased to 0.40% or decreased about 41.0% from M0 generation.

(b) CBD content. It was found that average content of CBD of male and female plants of M0 generation were 0.41% and 0.34%, respectively, averaged 0.36% of both sexes. In M1 generation, CBD content of male and female plants increased to 0.42% and 0.73%, respectively, average of both sexes increased to 0.63% or increased about 75.0% from M0 generation.

B. Chemotype classification:

(a) Non-drug type. Results indicated that M0 generation consisted of 14.0% of male and 14.0% of female plants, averaged 14.0% of both sexes. In M1 generation, average of male and female plants increased to 45.8% and 42.0%, respectively, average of both sexes increased to 43.9% or about 214.0% increased from M0 generation. There was high positive significant relationship between THC and CBD content for this chemotype group with $R^2=0.91^{**}$.

(b) Intermediate type. Results indicated that M0 generation consisted of 26.0% of male and 15.0% of female plants, averaged 20.5% of both sexes. In M1 generation, male plants decreased to 20.8% but female plants increased to 37.0%, average of both sexes increased to 28.9% or about 41.0% increased from M0 generation. There was high positive significant relationship between THC and CBD content in this chemotype as well with $R^2=0.90^{**}$.

(c) Drug type. It was found that M0 generation consisted of 60.0% of male and 71.0% of female plants, averaged 65.5% of both sexes. In M1 generation, male and female plants decreased to 33.4% and 21.0%, respectively, average of both sexes decreased to 27.2% or about 58.0% decreased from M0 generation. There was high positive significant relationship between THC and CBD content of this chemotype as well with $R^2=0.72^{**}$.

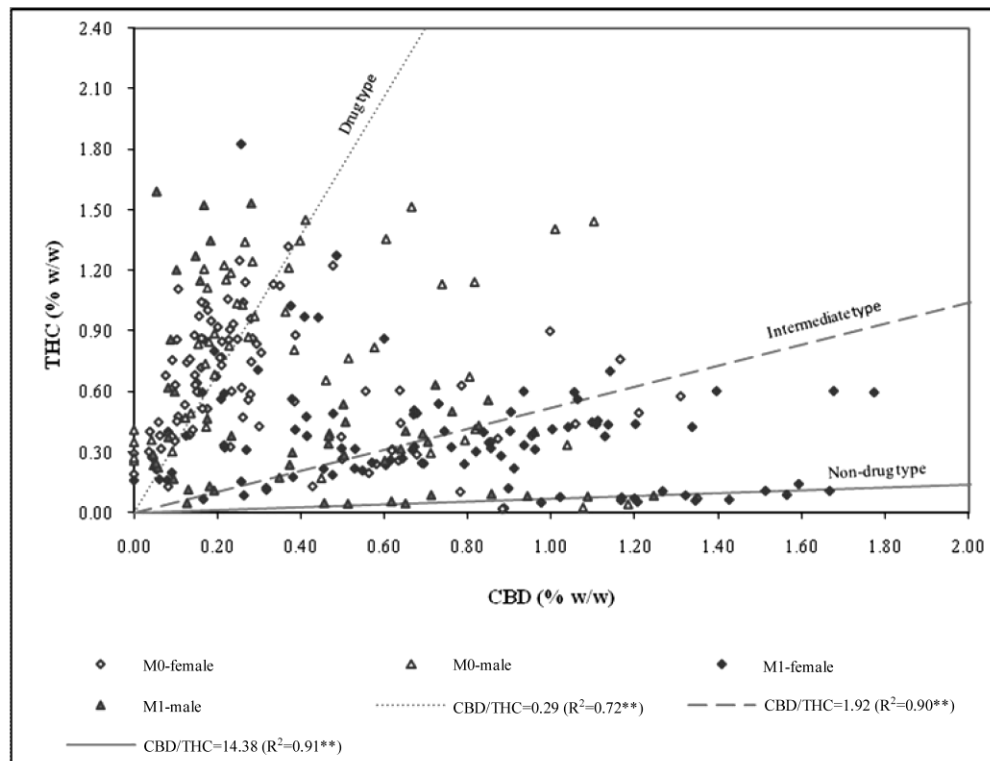


Figure 2. THC and CBD contents of individual plant belongs to M0 and M1 of Mae Sa Mai hemp cultivar and relationship between THC and CBD of each chemotype group. The experiment was conducted at Pangda Agricultural Station in 2008 and 2009 growing seasons.

Table 2. THC content, CBD content and chemotype group of M0 and M1 of Mae Sa Mai hemp cultivar.

Chemotype content/group	M0			M1		
	Male	Female	Average	Male	Female	Average*
A) Chemotype content						
a) THC content (%)	0.79	0.62	0.68	0.45	0.38	0.40 (-41%)
b) CBD content (%)	0.41	0.34	0.36	0.42	0.73	0.63 (+75%)
B) Chemotype group						
a) Non-drug type (%)	14.0	14.0	14.0	45.8	42.0	43.9 (+214%)
b) Intermediate type (%)	26.0	15.0	20.5	20.8	37.0	28.9 (+41%)
c) Drug type (%)	60.0	71.0	65.5	33.4	21.0	27.2 (-58%)

* Values in brackets are percentages of increasing or decreasing chemotype content and chemotype groups.

3. Huay Hoi cultivar.

A. Chemotype composition:

(a) THC content. Results indicated that average THC content of male and female plants of M0 generation were 0.54% and 0.47%, respectively, averaged 0.49% of both sexes. In M1 generation, average THC content of male and female plants decreased to 0.45% and 0.37%, respectively, average of both sexes decreased to 0.40% or about 18.0% decreased from M0 generation.

(b) CBD content. It was found that average CBD content of M0 generation of male and female plants were 0.45% and 0.34%, respectively, average of both sexes was 0.38%. In M1 generation, CBD content of male plants decreased to 0.43% but female plants increased to 0.60%, average value of both sexes increased to 0.55% or increased about 45.0% from M0 generation.

B. Chemotype classification:

(a) Non-drug type. Results indicated that average non-drug type of M0 generation consisted of 32.0% of male and 38.0% of female plants, averaged 35.0% of both sexes. In M1 generation, average of male and female plants increased to 43.6% and 50.0%, respectively, average of both sexes increased to 46.8% or increased about 34.0% from M0 generation. There was high positive significant relationship between THC and CBD content in this chemotype group with $R^2=0.84^{**}$.

(b) Intermediate type. Results indicated that average intermediate type of M0 generation consisted of 24.0% of male plants and 6.0% of female plants; average of both sexes was 15.0%. In M1 generation, average of male and female plants increased to 30.8% and 21.1%, respectively, average of both sexes increased slightly to 26.0% or about 73.0% increased from M0 generation. There was a high positive significant relationship between THC and CBD content of this chemotype group as well with $R^2=0.92^{**}$.

(c) Drug type. It was found that drug type of M0 generation consisted of 44.0% of male plants and 56.0% of female plants, averaged 50.0% of both sexes. In M1 generation, average of male and female plants decreased to 25.6% and 28.9%, respectively, average of both sexes decreased to 27.2% or about 46.0% decreased from M0 generation. There was high positive significant relationship between THC and CBD content for this chemotype group with $R^2=0.77^{**}$.

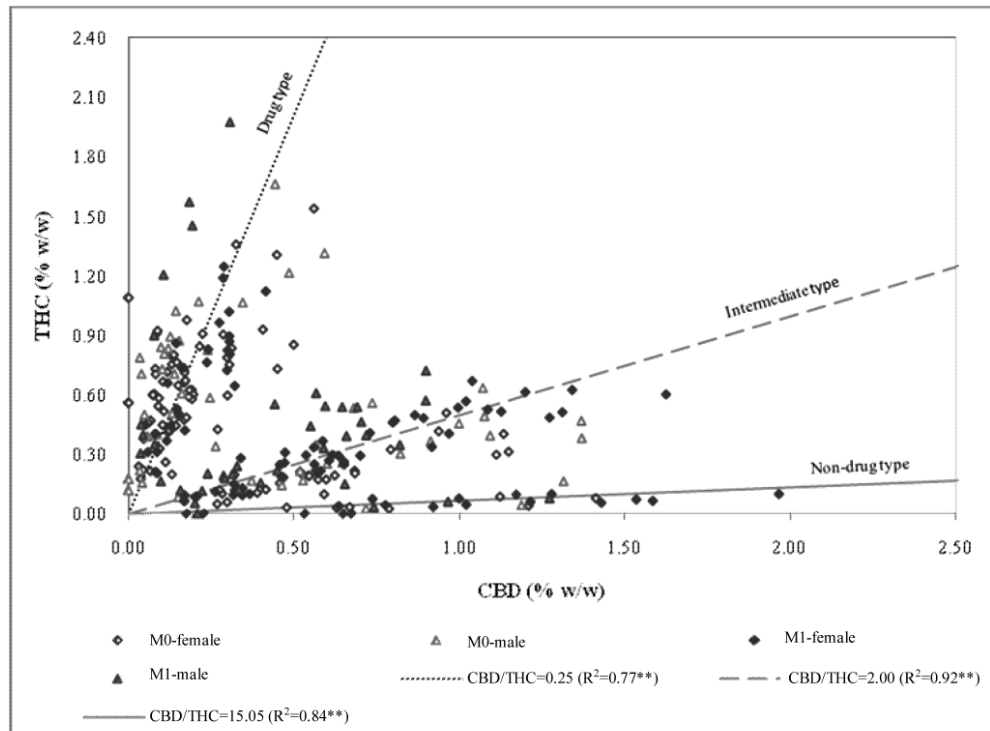


Figure 3. THC and CBD contents of individual plant belongs to M0 and M1 of Huay Hoi hemp cultivar and relationship between THC and CBD of each chemotype group. The experiment was conducted at Pangda Agricultural Station in 2008 and 2009 growing seasons.

Table 3. THC content, CBD content and chemotype group of M0 and M1 of Huay Hoi hemp cultivar.

Chemotype content/group	M0			M1		
	Male	Female	Average	Male	Female	Average*
A) Chemotype content						
a) THC content (%)	0.54	0.47	0.49	0.45	0.37	0.40 (-18%)
b) CBD content (%)	0.45	0.34	0.38	0.43	0.60	0.55 (+45%)
B) Chemotype group						
a) Non-drug type (%)	32.0	38.0	35.0	43.6	50.0	46.8 (+34%)
b) Intermediate type (%)	24.0	6.0	15.0	30.8	21.1	26.0 (+73%)
c) Drug type (%)	44.0	56.0	50.0	25.6	28.9	27.2 (-46%)

* Values in brackets are percentages of increasing or decreasing chemotype content and chemotype groups.

4. Pang Ung cultivar.

A. Chemotype composition:

(a) THC content. The results indicated that average THC content of male and female plants of M0 generation was 0.70% and 0.71%, respectively, averaged 0.71% of both sexes. In M1 generation, average THC content of male and female plants decreased to 0.65% and 0.51%, respectively, average of both sexes decreased to 0.55% or decreased about 23.0% from M0 generation.

(b) CBD content. It was found that average CBD content of M0 generation of male and female plants were 0.16% and 0.15%, respectively, average of both sexes was 0.15%. In M1 generation, average CBD content of male and female plants increased to 0.35% and 0.34%, respectively, average value of both sexes increased to 0.34% or increased about 127.0% from M0 generation.

B. Chemotype classification:

(a) Non-drug type. Results indicated that average non-drug type of M0 generation consisted of 18.0% of male plants and 18.0% of female plants, averaged 18.0% of both sexes. In M1 generation, average of male and female of non-drug type increased to 25.5% and 26.5%, respectively, average of both sexes increased to 26.0% or increased about 44.0% from M0 generation. For this chemotype group, association between THC and CBD content could not be identified.

(b) Intermediate type. Results indicated that average intermediate type of M0 generation consisted of 4.0% of male plants and 1.0% of female plants, averaged 2.5% of both sexes. In M1 generation, average of male and female plants increased to 29.8% and 18.4%, respectively. Average of both sexes increased to 24.1% or increased about 864.0% from M0 generation. There was high positive significant relationship between THC and CBD content of this chemotype group with $R^2=0.88^{**}$.

(c) Drug type. It was found that drug type of M0 generation consisted of 78.0% of male plants and 81.0% of female plants, averaged 79.5% of both sexes. In M1 generation, average of male and female plants decreased to 44.7% and 55.1%, respectively, average of both sexes decreased to 49.9% or decreased about 37.0% from M0 generation. There was high positive significant relationship between THC and CBD content of this chemotype group as well with $R^2=0.79^{**}$.

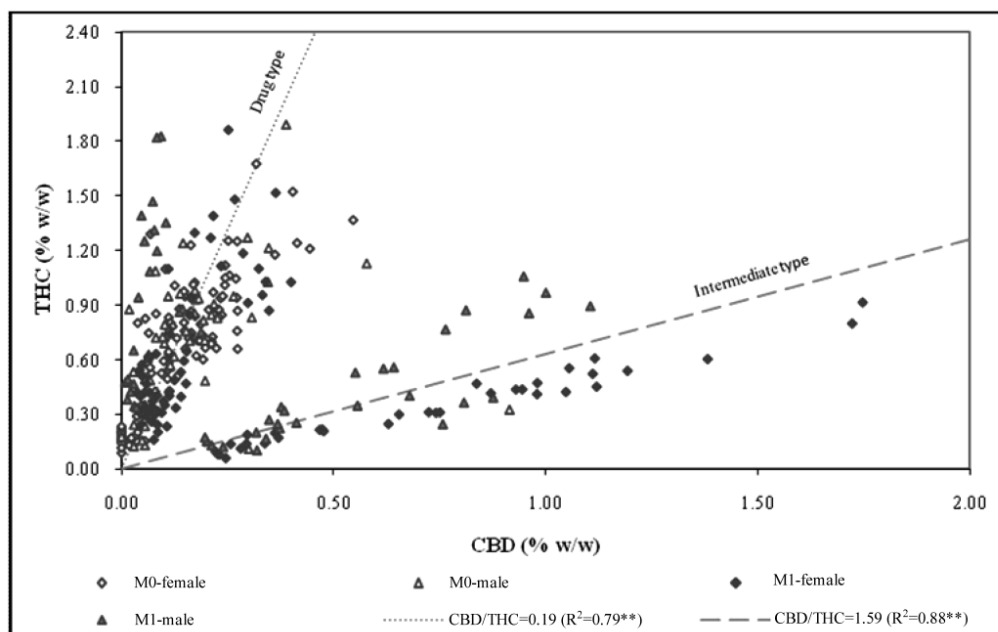


Figure 4. THC and CBD contents of individual plant belongs to M0 and M1 of Pang Ung hemp cultivar and relationship between THC and CBD of each chemotype group. The experiment was conducted at Pangda Agricultural Station in 2008 and 2009 growing seasons.

Table 4. THC content, CBD content and chemotype group of M0 and M1 of Pang Ung hemp cultivar.

Chemotype content/group	M0			M1		
	Male	Female	Average	Male	Female	Average*
A) Chemotype content						
a) THC content (%)	0.70	0.71	0.71	0.65	0.51	0.55 (-23%)
b) CBD content (%)	0.16	0.15	0.15	0.35	0.34	0.34 (+127%)
B) Chemotype group						
a) Non-drug type (%)	18.0	18.0	18.0	25.5	26.5	26.0 (+44%)
b) Intermediate type (%)	4.0	1.0	2.5	29.8	18.4	24.1 (+864%)
c) Drug type (%)	78.0	81.0	79.5	44.7	55.1	49.9 (-37%)

* Values in brackets are percentages of increasing or decreasing chemotype content and chemotype groups.

DISCUSSION

Improvement of hemp crop for reduced THC content by using mass selection method was carried out during 2008-2009 cropping seasons. After one cycle of selection in both male and female plants, results indicated that local hemp cultivars which were grown on the highland areas in Thailand had a wide range of variation in THC and CBD contents. The variation in content of these two chemotypes could be classified clearly into three chemotype groups which are: (1) drug type (CBD/THC <1.0); (2) intermediate type ($1.0 \leq \text{CBD/THC} \leq 10.0$); and (3) non-drug type (CBD/THC >10.0). These results were similar to those reported by de Meijer *et al.* (1992, 2003), Hillig and Mahlberg (2004), Mandolino and Carboni (2004) and Mechtler *et al.* (2004).

Reduction of THC and increasing CBD content of each hemp varietal population was also examined after one generation of selection. These results were obtained since the number of plants belonging to non-drug and intermediate types were increased and vice versa for drug type plants. In addition, using of mass selection method is an effective and appropriate means for selecting qualitative traits which are controlled by few genes (Allard, 1960). Progress in selection for low THC content in local hemp cultivars is mainly due to genetic factors which THC and CBD traits are controlled by single locus of gene with two co-dominant alleles (de Meijer *et al.*, 2003; Mandolino *et al.*, 2003). As well, heritability of THC content is rather high (Kunkaew *et al.*, 2010).

This study evidently revealed that there were high positive significant relationships between THC and CBD content of three chemotype groups (drug, intermediate and non-drug) of four hemp cultivars with R^2 ranging from 72.0-92.0%; these similar results were reported by Hennink (1994). Thus, selection for reduced THC content, high ratio of CBD/THC content could be used as an alternative criterion for improving low THC content in hemp cultivars. It is anticipated that low THC content hemp cultivars with good agronomic characters will be obtained from this research project and are suitable for agricultural production by farmers who live on the tropical highland areas in the northern part of Thailand.

ACKNOWLEDGEMENTS

The authors would like to thank Royal Project Foundation and Highland Research and Development Institute (Public Organization) for supporting to conduct this experiment.

REFERENCES

- Allard, R.W. 1960. Principles of plant breeding. John Wiley & Sons, Inc. London, 485 p.
- Amaducci, S., M. Colauzzi, A. Zatta and G. Venturi. 2008. Flowering dynamics in monoecious and dioecious hemp genotypes. *J. Ind. Hemp* 13: 5-19.
- Callaway, J.C. 2008. A more reliable evaluation of hemp THC levels is necessary and possible. *J. Ind. Hemp* 13: 117-144.
- De Meijer, E.P.M. and L.J.M. van Soest. 1992. The CPRO *Cannabis* germplasm collection. *Euphytica* 62: 201-211.
- De Meijer, E.P.M., H.J. van der Kamp and F.A. van Eeuwijk. 1992. Characterisation of *Cannabis* accessions with regard to cannabinoid content in relation to other plant characters. *Euphytica* 62: 187-200.
- De Meijer, E.P.M., M. Bagatta, A. Carboni, P. Crucitti, V.M.C. Moliterni, P. Ranalli and G. Mandolino. 2003. The inheritance of chemical phenotype in *Cannabis sativa* L. *Genetics* 163: 335-346.
- Hennink, S. 1994. Optimisation of breeding for agronomic traits in fiber hemp (*Cannabis sativa* L.) by study of parent-offspring relationships. *Euphytica* 78: 69-76.
- Hillig, K.W. and P.G. Mahlberg. 2004. A chemotaxonomic analysis of cannabinoid variation in *Cannabis* (Cannabaceae). *American Journal of Botany* 91: 966-975.
- Jobling, T. and P. Warner. 2001. New tropical industrial hemp. Proceeding of the 10th Australian Agronomy Conference 2001, Australian Society of Agronomy. <http://www.regional.org.au/au/asa/2001/5/c/jobling.htm#TopOfPage>
- Johnson, R. 2010. Hemp as an agricultural commodity. Congressional Research Service. Report for Congress (Code No. RL32725). 22 p.
- Kaveeta, L., Y. Paopun, K. Promratrak, M. Nanakorn, S. Suwanwong, R. Sengloung, W. Nanakorn and S. Thativivat. 2006. Morphological and anatomical characteristics of hemp (*Cannabis sativa* L.) in Thailand. (in Thai, with English abstract) *Agricultural Science Journal* 37(4): 293-302.
- Kunkaew, W., S. Julsrigival, P. Tipparat, A. Punyalue, V. Punsupa, M. Srihawong and P. Auntin. 2009. Classification of hemp (*Cannabis indica* L.) in Thailand by THC and CBD content. (in Thai, with English abstract) *Agricultural Science Journal* 40: 307-314.
- Kunkaew, W., S. Julsrigival, P. Tipparat, V. Punsupa and P. Auntin. 2010. Heritability of delta-9-tetrahydrocannabinol contents in hemp (*Cannabis indica* L.) grown on highland area in Thailand. (in Thai, with English abstract) *Agricultural Science Journal* 41: 75-80.
- Mandolino, G. and A. Carboni. 2004. Potential of marker-assisted selection in hemp genetic improvement. *Euphytica* 140: 107-120.
- Mandolino, G., M. Bagatta, A. Carboni, P. Ranalli and E. de Meijer. 2003. Qualitative and quantitative aspects of the inheritance of chemical phenotype in *Cannabis*. *J. Ind. Hemp* 8: 51-72.
- Mechtler, K., J. Bailer and K. de Hueber. 2004. Variation of Δ^9 -THC content in single plants of hemp varieties. *Industrial Crops and Products* 19: 19-24.
- Ranalli, P. 1999. Advances in hemp research. Food Products Press (Haworth Press), London, 272 p.

- Sengloung, T., L. Kaveeta and W. Nanakorn. 2009. Effect of sowing date on growth and development of Thai hemp (*Cannabis sativa* L.). *Kasetsart J. (Nat. Sci.)* 43: 423-431.
- Steel, R.G.D. and G.H. Torrie. 1960. Principles and procedures of statistics. Mc. Graw Hill Book Comp. Inc. New York, 481 p.
- United Nations. 1987. Recommended methods for testing cannabis. New York.