Executive summary

Title Development of Medicinal Plants

Sub-project 1

Genetic Diversity of Plai (*Zingiber montanum* (Koen.) Theilade) in Thailand

Sub-project 2

Genetic Diversity of Wan Chak Mod Loog (*Curcuma comosa* Roxb., *Curcuma latifolia* and *Curcuma xanthorhiza* Roxb.) in Thailand

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Budget year 2009 Budget amount 5,233,000 Baht

Duration: May 2009 - May 2010

Background/Problem

Plai and Wan Chak Mod Loog are two of the commonly used herbal medicines in Thailand. These medicinal plants are cultivated for economic purposes, but the knowledge involving an identification of plant varieties is still limited. Although there were some reports indicating an identification of Plai and Waan Chak Mod Loog via RAPD technique, the number of samples collected and the morphological data were insufficient. Moreover, because of the occasional unreliability and unrepeatability of PCR results, the RAPD methodology has faced with legitimate criticism, thus the process of identifying the varieties of Plai and Waan Chak Mod Loog is still unclear.

The objective of this research project is to identify the varieties of Plai and Wan Chak Mod Loog using AFLP technique, since this technique is more reliable than RAPD. We also have high potential to collect samples throughout Thailand, confirming that the project will be providing a highly efficient and reliable result in varieties identification. After the varieties identification result was obtained, we can then select some promising varieties for more experiments on yield testing, oil quantity testing, and biological-active composition testing. The promising varieties, which have high yield or high level of biological active compounds, will be introduced to farmer; accordingly, these varieties can increase yield and reduce production cost. In the near future, the varieties identification data will be quite useful for other research fields such as pharmacology and toxicology.

Objectives

- To evaluate the genetic variation of Plai and Wan Chak Mod Loog.
- To identify Plai varieties using DNA fingerprint generated by AFLP technique, morphological characteristics and level of volatile oil.
- To select Plai varieties that provide high yield and high volatile oil.
- To identify Wan Chak Mod Loog varieties using DNA fingerprint generated by AFLP technique along with morphological characteristics.

Methodology

Sub-project 1 Genetic Diversity of Plai (*Zingiber montanum* (Koen.) Theilade) in Thailand

The objectives of this sub-project are (1) to find the appropriate method of volatile oil extraction; (2) to evaluate genetic variation and to identify Plai varieties using AFLP technique and (3) to select Plai varieties providing high yield and high volatile oil quality.

The experiment on volatile oil extraction, the water distillation and hexane extraction methods were studied. Chemical compounds of volatile oil extracted from each method were analyzed by GC-MS. Namely, three samples and three replications of the extractions and analysis procedures were performed for each of the run. The extraction efficiencies and chemical compounds of volatile oil, defining the result conducted via different methods, were investigated. The chemical compound results were compared with the standards defined by Thai Industrial Standard Institute and Thailand Institute of Scientific and Technological Research. In essence, the most appropriate extraction method was selected and then used to extract volatile oil, a product proper for utilizing in further experiment.

The research on evaluation of genetic variation and variety identification of Plai was divided into two experiments. The first experiment was performed in Plai group 1 containing 21 samples, which were planted for 32 months. Wherefore, the second experiment was carried out in Plai group 2 containing 132 samples, which have been planted in 2009. The total genomic DNA was extracted from each sample and then subjected to AFLP fingerprinting.

In Plai group 1, the morphological characters and rhizome yields were also observed. The volatile oil of each sample was extracted using the appropriate method and the chemical compound results were compared with the standards described in the above experiment. The DNA fingerprint data, along with the morphological data, were used for the varieties identification of Plai group 1. In Plai group 2, the identification of Plai varieties only depended on AFLP fingerprint data.

Sub-project 2 Genetic Diversity of Wan Chak Mod Loog (*Curcuma comosa* Roxb., *Curcuma latifolia* and *Curcuma xanthorhiza* Roxb.) in Thailand

A total of 411 accessions of *Curcuma* spp. was collected from cultivated sites throughout Thailand. Sixty samples, which showed different phenotypes, were selected. Total genomic DNA was extracted and then subjected to AFLP fingerprinting. In addition, the morphological characters were observed. The DNA fingerprint data, along with the morphological data, was used for the varieties identification of Wan Chak Mod Loog.

Results

Sub-project 1 Genetic Diversity of Plai (*Zingiber montanum* (Koen.) Theilade) in Thailand

This sub-project consists of three main experiments; (1) the study on volatile oil extraction methods; (2) the evaluation of genetic variation and variety identification of Plai using AFLP technique and (3) the selection of Plai varieties providing high yield and high volatile oil quality.

To come up with an appropriate method on volatile oil extraction, the water distillation and hexane extraction were studied. The result indicated that water distillation showed high extraction efficiency in term of quantity, physical characteristics, and chemical compositions. We concluded that the best approach for volatile oil extraction from Plai rhizome was water distillation, which will be used in our further experiment.

The second experiment was performed to evaluate the genetic variation and to identify varieties of Plai using AFLP technique. This research was divided into two sub-experiments. The first sub-experiment was performed in Plai group 1 containing 21 samples, which were planted for 32 months, and the second sub-experiment was carried out in Plai group 2 containing 132 samples, which have been planted in 2009. The varieties identification result based on AFLP fingerprinting showed that Plai group 1 containing 21 samples could be divided into six varieties. These groupings are relevant to some morphological differences of the genotype such as white-stripe or yellow-strip leaf, inflorescence shapes and the height of pseudostem. Those morphological data along with AFLP fingerprint data can be use for selection of Plai varieties providing high yield and high volatile oil in the future.

The last part was the selection of promising variety providing high yield and high volatile oil quality. When considering the volatile oil yield, the fifth variety showed the highest average volatile oil yield per clump at 88.1 ml/clump and the second average volatile oil yield was 63.3 ml/clump found in the first variety. We suggest that further experiment on yield test of all varieties should be focused to confirm this result. The morphological characteristics, rhizome yield and volatile oil yield of Plai group 2 should be also observed in next year when it matures enough to harvest rhizomes.

The genetic similarity between cassumunar gingers assessed by AFLP markers revealed that there were several duplicate accessions in the germplasm collection. The identification of Plai group 1 varieties showed that 21 samples were reduced to six varieties, whereas samples in Plai group 2 can be reduced from 132 samples to 49 varieties.

Sub-project 2 Genetic Diversity of Wan Chak Mod Loog (*Curcuma comosa* Roxb., *Curcuma latifolia* and *Curcuma xanthorhiza* Roxb.) in Thailand

A total of 411 accessions of Curcuma spp. was collected from cultivated sites throughout Thailand. Sixty samples which showed different phenotypes were selected and then subjected to AFLP fingerprinting. The result indicated that Wan Chak Mod Loog samples could be divided into three groups. Group I and group III were defined as Wan Chak Mod Loog based on previous reference reports. The samples in group III could be assigned to *Curcuma comosa*. The samples in group I and the related species samples which clustered together into group II could be assigned to *Curcuma* sp. We found that the morphological characteristics and AFLP data could be used to accurately identify and classify all sixty samples.

Recommendations

The results of this project showed that AFLP technique can be successfully applied to identify Plai and Waan Chak Mod Loog varieties. Interestingly, the samples within the same cluster shared some phenotypic characteristics. We suggested that the thoroughly investigation on morphology of each variety should be performed and then using morphological data along with DNA fingerprint data to accurately identify and classify plant varieties. Moreover, the morphological data are also useful for taxonomic study.

The result of Plai group 1 indicating that the average volatile oil yield of the fifth variety was significantly higher than other varieties. We recommend that further experiment on yield test of all varieties should be focused to confirm this result. We suggest that the morphological characteristics, rhizome yield and volatile oil yield of Plai group 2 should be observed in next year when it matures enough to harvest rhizomes.

In Wan Chak Mod Loog, the result revealed that sixty samples could be divided into three groups. The different genotypes of Wan Chak Mod Loog might contain various levels of biologically active compounds. Further investigation should focus on the pharmacological and toxicological characterization of the related species and the various genotypes of Wan Chak Mod Loog.

Utilities

In the current study, the AFLP technique has proven to be useful in investigating the genetic variation of Plai and Wan Chak Mod Loog. The agreement observed between the AFLP-based dendrogram and the classification based on morphological characters also proved that AFLPs can be successfully used to study genetic relationships. The AFLP technique can be applied to other experiments on identification or classification of medical herbs.

The redundant cost of field maintenance caused by duplicate accessions is an important problem in large collections of germplasm. The genetic similarity between Plai samples assessed by AFLP markers revealed that there were several duplicate accessions in the germplasm collection so we can reduce cost of field maintenance by get rid of the unnecessary accessions.

After the varieties identification result of Plai group 1 was obtained, we can select some promising varieties for more experiments on yield testing, oil quantity testing and biological-active composition testing. The fifth variety, showed high average volatile oil yield, was recommended as promising variety. The yield testing of this variety will be investigated and this variety may be introduced to farmer. The elite variety can increase yield and reduce production cost.

In Plai group 2, the germplasm contains more than 100 samples which is now the most extensive collection and genetic relationship among them was assessed as a first step towards their genetic improvement. This germplasm collection was a valuable source of promising raw material for further crop improvement.

Previously, the identification of Wan Chak Mod Loog has been ambiguous. Misidentification of this herbal plant can lead to its substitution with potentially toxic plants. The research of Wan Chak Mod Loog in this project provides useful data on identification and classification of this medicinal herb and also provides some key morphological traits that could be used to distinguish some *Curcuma* species. Moreover, the species and variety identification data from this project will be very useful for other researches such as pharmacology and toxicology in the future.

Abstract

This research project aims to evaluate the genetic diversity and to identify varieties of two medicinal herbs, Plai and Wan Chak Mod Loog. These herbs belong to Zingiberaceae family which the knowledge involving an identification of species and varieties is still unclear. In the present study, the AFLP marker was used for variety identification of those herbs. The study divided into two sub-project; (1) Genetic Diversity of Plai (*Zingiber montanum* (Koen.) Theilade) in Thailand and (2) Genetic Diversity of Wan Chak Mod loog (*Curcuma comosa* Roxb., *Curcuma latifolia* and *Curcuma xanthorhiza* Roxb.) in Thailand.

Sub-project 1 Genetic Diversity of Plai (*Zingiber montanum* (Koen.) Theilade) in Thailand

Sub-project 1 is the study of genetic diversity of Plai in Thailand. This sub-project consists of three main experiments; (1) the study on volatile oil extraction methods; (2) the evaluation of genetic variation and the variety identification of Plai using AFLP technique and (3) the selection of promising variety providing high volatile oil yield.

The first part is the experiment to find the appropriate method for Plai volatile oil extraction. The water distillation and hexane extraction methods were studied. The result indicated that water distillation showed high extraction efficiency in term of quantity, physical characteristics, and chemical compositions. We concluded that the best approach for volatile oil extraction from Plai rhizome was water distillation, which will be used in our further experiment.

The second part was performed to evaluate the genetic diversity and to identify varieties of Plai using AFLP technique. This research was divided into two sub-experiments. The first sub-experiment was performed in Plai group 1 containing 21 samples, which were planted for 32 months, and the second sub-experiment was carried out in Plai group 2 containing 132 samples, which have been planted in 2009. The result showed that AFLP technique can be successfully applied to identify Plai varieties. The AFLP fingerprints revealed that Plai group 1 could be divided into six varieties and Plai group 2 could be divided into 49 varieties. These groupings were also relevant to some morphological differences of the genotypes such as white-stripe or yellow-strip leaf, inflorescence shapes and the height of pseudostem.

The last part, we investigated the volatile oil yields of Plai group 1 in order to select the promising variety. The result revealed that the fifth variety showed the highest average volatile oil yield per clump at 88.1 ml and the second average volatile oil yield was 63.3 ml found in the first variety. We recommended the fifth variety as a promising variety. We suggest that further experiment on yield test of all varieties should be focused to confirm this result. The morphological characteristics, rhizome yield and volatile oil yield of Plai group 2 should be also observed in next year when it matures enough to harvest rhizomes.

Sub-project 2 Genetic Diversity of Wan Chak Mod Loog (*Curcuma comosa* Roxb., *Curcuma latifolia* and *Curcuma xanthorhiza* Roxb.) in Thailand

This study was performed to assess genetic diversity and to identify varieties of Wan Chak Mod Loog using AFLP technique. In total, 411 accessions of *Curcuma* spp. were collected from cultivated sites throughout Thailand. Sixty samples which showed different phenotypes were selected and then subjected to AFLP fingerprinting. The result indicated that all 60 samples could be divided into three groups. Considering the morphological characteristics, the samples in group III could be assigned to *Curcuma comosa*, whereas samples in group I and related species samples which clustered in group II could be assigned to *Curcuma* sp. We found that the morphological characteristics and AFLP data could be used to accurately identify and classify all sixty samples.

Keywords: medicinal herb, *Zingiber montanum*, *Curcuma comosa*, volatile oil extraction, chemical composition, DNA marker, AFLP, genetic diversity