บันทึกข้อความ

ส่วนราชการ.....คณะวิทยาศาสตร์และเทคโนโลยี...มหาวิทยาลัยราชภัฏเพชรบูรณ์ .....(นางสาววนิดา...ภัคล่อรุ่ง)

ที่....วศ ๐๗ / ๕๙.....**วันที่**..... ๒ มีนาคม ๒๕๕๙.....

เรื่อง ขอส่งบทความทางวิชาการที่นำเสนองานประชุมวิชาการระดับนานาชาติ

เรียน คณบดีคณะวิทยาศาสตร์และเทคโนโลยี

ตามที่ ข้าพเจ้าได้รับการอนุญาตให้นำเสนองานวิจัย เรื่อง DNA Barcodes for Cultivar Identification of Thailand *Tamarindus indica* L. ในการประชุมวิชาการนานาชาติ " The 2015 International Conferrence on Science and Technology (TICST 2015)" ซึ่งจัดเมื่อ วันที่ ๔ – ๖ พฤศจิกายน ๒๕๕๘ ณ มหาวิทยาลัยราชมงคลเทคโนโลยีธัญญบุรี ถนนรังสิต-นครนายก อำเภอธัญญบุรี จังหวัดปทุมธานี ความละเอียดแจ้งแล้วนั้น

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DNA Barcodes for cultivar identification of Thailand *Tamarindus indica*.L.

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***Abstract*– This study aims to investigated *Tamarindus indica.*L. chloroplast *rbcL* gene for ribulose-1,5-bisphosphate carboxylase/oxygenase, large subunit as the molecular marker to identified the cultivar level of *Tamarindus indica.*L. in Thailand. The partial *rbcL* gene of 19cultivars were sequences approximately 611-712 bp. The sequence alignment and Phylogenetic Tree analysis were generated by Clustal W and NJ methods. The result of nucleotide sequence alignment of partial *rbcL* gene for all cultivars were divided into 19 groups. The different of these groups similar to the different of local name of Tamarind cultivars in Phetchabun Province, Thailand. Base on these results indicated that 19 cultivar DNA Barcodes can supported the local opinions act as the scientific data to identification of tamarind cultivars in Thailand.**

***Keywords*–DNA Barcodes, *Tamarindus indica* L., *RbcL* gene, Ribulose-1,5-bisphosphate carboxylase/oxygenase, Rubisco.**

I. INTRODUCTION

Tamarinds are the symbol and economic agricultural plant products of Phetchabun Province, Thailand due to their usefulness. There are so many cultivars of Tamarinds in Thailand with the same scientific name: *Tamarindus indica* L. such as: Prakaitong, Srichompoopling, Muenjong, Sangartit, Srichompooklom, Preao–puenmuang, Sripakdee, Phetbourtong, Preao – yak, Khuntee soy, Praroj, Khuntee yourk, Numpueng etc. These are named by the farmers and the merchants called. So that the same cultivar had two or three names. Confusion were happened in the using purpose for food or medicinal. Another confusion was planting purpose as too many young tamarind plants were faked. Furthermore, the world climate is changed, the tamarinds in Thailand are contaminated with fungi. The complete automated procedure for systematic , crop improvement or gene variation studying is necessary. Molecular data base is performed as DNA Barcodes. These research project aims to reconstruction Phylogenetic Tree.
Simultaneously, single nucleotide is analyzed for using as molecular marker to diagnosis, classification and cultivar identification for tamarind crop improvement in Thailand.

II. MATHERIAL AND METHOD

A. Samples

Around 1,900 tamarind seeds of different cultivars were used in this study. The 7 day leaves of seedling were collected and kept at -20 degree celsius for DNA analysis. The 19 cultivars of *Tamarindus indica* L. were : Preao–yak;(PRY), Sangartit;(SAT),

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Praroj;(PRR), Prakaiphet;(PKP), Prakaitong;(PKT), Khuntee –yourk;(KTY), Khuntee –soy;(KTS),

Seetongbow;(STB), Seetongnug; (STN), Fagdap;(FAD),Numpueng;(NUP), Muenjong; (MUJ), Seetongjook;(STJ), Sripakdee;(SPD), Intapalum;(ITPL), Preaopuenmuang;(PPM), Phetbourtong; (PBT), Srichompootongpling; (SCPP), and Srichompootongklom;(SCPK). As show in Fig.1 and 2.

B. DNA extractions

DNA extractions and normalization were performed by C-TAB method[1], using Real Genomics Kit,RBC Bioscience Crop,Taiwan.

C. PCR amplification and detection

1) PCR amplifications are performed using 2 primers which were designed by Macrogen Ltd., Korea. The two primers are RbcL-Tam-F03 and RbcL-Tam-R04.[2]. PCR products were detected through 1% gel electrophoresis and send to sequencing

2) The DNA sequencing of 19 tamarinds were aligned by Clustal W (Fig.3) and using NJ method for phylogenetic tree reconstruction[3,4].As shown in Fig.4

3) The single nucleotide of each cultivar was analyzed using BLASTn, compared to *T.indica rbcL* gene from GenBank (Acession No : AB378727 gi 219687663)[7].].As shown in Table1.

III. RESULTS AND DISSCUSION

The results of sequencing and analization were shown in table 1 indicated that all of tamarinds were 611-712 bp.They were in the same species, concordance with the maxidentity(%)table, there were difference from each other not more than 5% (not show the data table)[5]

TABLE 1. The length of nucleotide sequences of *rbcL* gene from 19 Thai tamarind cultivars compared to *T.indica rbcL* gene from GenBank (Accession No : AB378727 gi 219687663) using Blastn v.2.2.27

19 Thailand Tamarind cultivars	**No. of bp (partial CDS)**	**Site to site of partial gene that compared to complete gene of T.*indica*L. *rbc*L gene. (complete CDS) 1…….1398**
1.Srichompooklom	641	…688…1346…
2.Srichompoopling	671	…744…1359…
3.Seetongbow	611	…731…1347…
4.Seetongnug	621	…731…1351…
5.Seetongjook	649	…704…1344…
6.Khuntee-yourk	643	…706…1347…
7.Khuntee-soy	649	…703…1349…
8.Sripakdee	678	…734…1386…
9.Sangartit	671	…692…1337…
10.Prakaiphet	646	…734…1372…
11.Preaopuenmuang	670	…700…1344…
12.Praroj	645	…706…1347…
13.Numpueng	712	…732…1394…
14.Intapalum	643	…706…1347…
15.Prakaitong	671	…729…1351…
16.Preao-yak	654	…704…1349…
17.Fagdap	644	…701…1350…
18.Muenjong	645	…699…1349…
19.Phetbourtong	657	…696…1349…

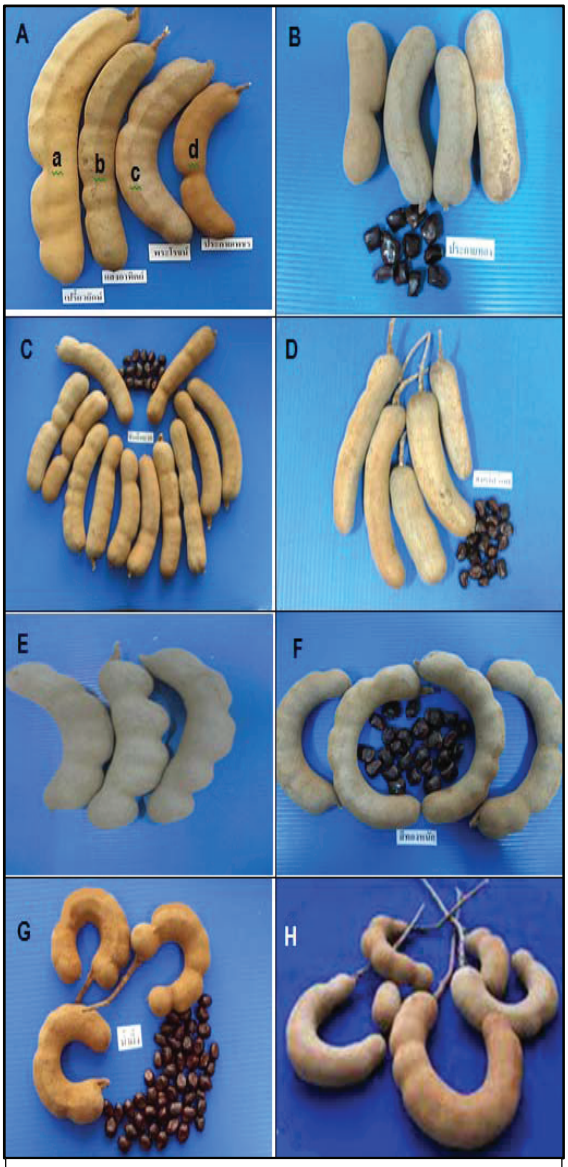

Figure 1: The feature of11ThaiTamarind Cultivars.

A. a= Preao–yak;(PRY),
b= Sangartit;(SAT),
c= Praroj;(PRR),
d= Prakaiphet;(PKP),
B.= Prakaitong;(PKT),
C.= Khunteeyourk;(KTY),
D.= Khuntee –soy;(KTS),
E.= Seetongbow;(STB),
F.= Numpueng;(NUP),
G.= Numpueng;(NUP),
H.= Muenjong; (MUJ),

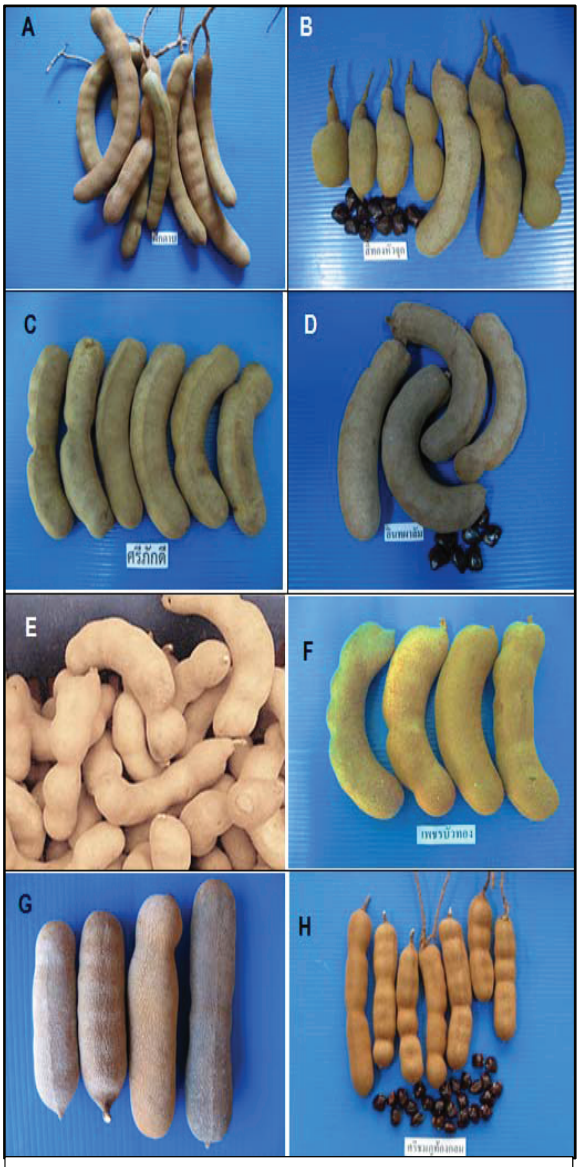

Figure 2 : The feature of 8 ThaiTamarind Cultivars.

A.= Fagdap;(FAD),
B.= Seetongjook;(STJ),
C.= Sripakdee;(SPD),
D.= Intapalum;(ITPL),
E.= Preao–puenmuang;(PPM),
F.= Phetbourtong; (PBT),
G.= Srichompootongpling;(SCPP),
H.= Srichompootongklom;(SCPK).

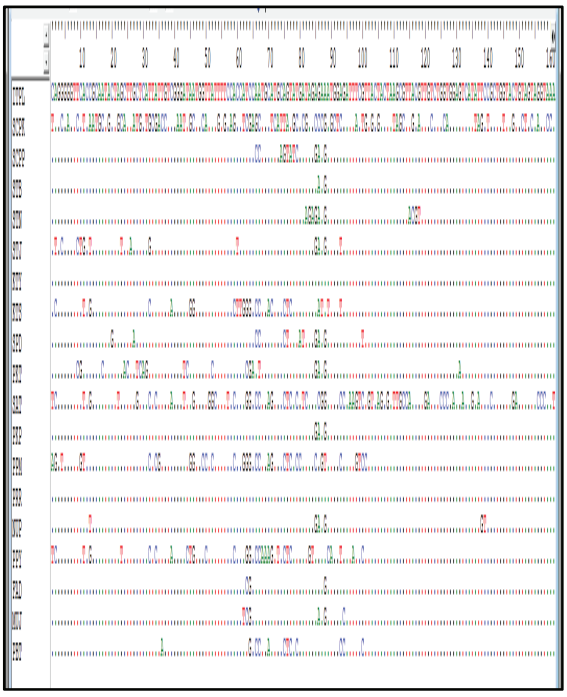

Figure 3 : Features and similarities in alignment window of 19 Tamarind cultivar sequences, present number of base 1-160 bp. DOT(.)=view conservation by plotting identities to a standard as a dot.

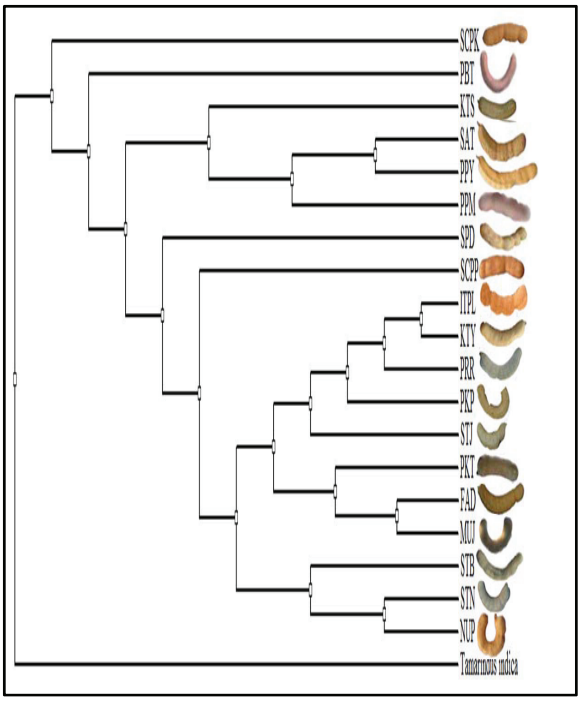

Figure 4 : Phylogenetic tree reconstruction from alignment of DNA sequencing in 19 cultivars of Tamarinds in Thailand. Using ***Tamarindus indica*** from GenBank as out group for rooting the tree trough NJ method.

SCPK = Srichompoo–tongklom;
P BT = Phetbourtong;
KTS = Khuntee–soy;
SAT = Sangartit;
PRY = Preao–yak;
PPM = Preao–puenmuang;
SPD = Sripakdee;
SCPP = Srichompoo–tongpling;
ITPL = Intapalum;
KTY = Khuntee–yourk;
PRR = Praroj;
PKP = Prakaiphet;
STJ = Seetongjook;
PKT = Prakaitong;
FAD = Fagdap;
MUJ = Muenjong;
STB = Seetongbow;
STN = Seetongnug;
NUP = Numpueng.

From Phylogenetic Tree of 19 Thailand tamarind cultivars in Fig. 4 could be devised into 15 groups, they were :

1. SCPK = Srichompoo–tongklom
2. PBT = Phetbourtong.
3. KTS = Khuntee–soy
4. SAT = Sangartit and PRY=Preao–yak
5. PPM = Preao–puenmuang
6. SPD = Sripakdee.
7. SCPP = Srichompoo–tongpling.
8. ITPL = Intapalum and KTY=Khuntee–yourk.
9. PRR = Praroj.
10. PKP = Prakaiphet.
11. STJ = Seetongjook.
12. PKT = Prakaitong.
13. FAD = Fagdap and MUJ=Muenjong.
14. STB = Seetongbow.
15. STN = Seetongnug and NUP=Numpueng

These data indicated that their single nucleotide sequences in partial of *rbcL* gene were variated or point mutated but the differences of each orther not over than 5%. So they are still in the same species. And they can be use as DNA Barcode for cultivar level identification of Thai Tamarinds.
Nevertheless, they have been concordanced with research of Patipanee khunthapok, 2007[6],

IV. CONCLUSION

1) From phylogenetic reconstruction study showed that the 19 Thailand tamarind cultivars were dividedinto 15 groups concordanced with the tamarind derived cultivars Phetchabun opinion in Phetchabun Province,Thailand.
2) *T.indica* Chloroplast *rbcL* gene for ribulose -1,5-bis phosphate carboxylaese /oxygenase large subunit, partial CDS only 611-712 bp, (while the full gene is

1398 bp.)enough for DNA Analysis. This result cause saving time and money.

3) These DNA barcodes could be used as molecular maker for classification and identification of *Tamarindus indica* L. in both species and cultivar levels. By comparison of single nucleotide sequences using Clustal W and Blatn software and can be use as DNA Barcodes for sustainably crop improvements and developments in Thailand.

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