Comparative Study of Proteome Pattern of Kluai Ta Nee and Kluai Nam Wa Leaf Proteins

Bung-on Prajanban[1]*, Wichuda Jankangram[2] and Nisachon Jangpromma[3,4]

[1]Faculty of Agricultural Technology, Burapha University, Sakaeo Campus, Sakaeo 27160, Thailand
[2]Faculty of Science and Social Sciences, Burapha University, Sakaeo Campus, Sakaeo 27160, Thailand
[3]Protein and Proteomics Research Center for Commercial and Industrial Purposes (ProCCI), Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
[4]Department of Integrated Science, Forensic Science Program, Faculty of Science, Khon Kaen University, Khon Kaen, 40002, Thailand.

*Corresponding author's e-mail: bungon.pr@buu.ac.th

Abstract:

This study compared the protein profiles in leaves of diploid and triploid banana to identify the differentially accumulated proteins unique to each type of banana. Protein samples were analyzed by 2D-PAGE using IPG strip pH3-10. Fifty and 44 protein spots were detected in leaves of *Musa acumimata* (BB group) "Kluai Ta Nee" and *Musa Xparadisiaca* (ABB group) "Kluai Nam Wa", respectively. Many spots of leaf proteins were corresponding to leaf proteins of *Musa acuminate* Colla (banana) reported previously such as ribulose-1,5-bisphosphate carboxylase, oxygen-evolving enhancer protein and superoxide dismutase. Ribulose-1,5-bisphosphate carboxylase, oxygen-evolving enhancer protein and superoxide dismutase in Kluai Ta Nee leaves had pIs and number of isoforms different from those in Kluai Nam Wa leaves. Three isoforms of endochitinase were found in Kluai Ta Nee leaves only. The major protein found in Kluai Ta Nee leaves, which had higher intensity than that in Kluai Nam Wa leaves, was identified as ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit. A low quality protein: oxygen-evolving enhancer protein 1, chloroplastic-like, found in Kluai Nam Wa leaves had higher intensity than that in Kluai Ta Nee leaves. The polymorphism of these proteins observed between leaves of Kluai Ta Nee and Kluai Nam Wa would be possibly due to different gene expressions in each banana type and may result in different functionalities of the proteins.

Keywords: 2D-PAGE, Kluai Ta Nee, Kluai Nam Wa, banana leaves

Introduction

Banana is one of the most important food crop of Musaceae family distributes in South East Asia, Africa and Australia [1]. Banana varieties grown in most parts of the world are hybrids derived from natural inter- and intraspecific crosses between two diploid wide species, *Musa acuminate* (genome A) and *Musa balbisiana* (genome B) [2]. Protein patterns can be used to study genetic diversity in banana. Proteins play a central role in biological processes, and proteomics differential assay can be used to determine the proteins that are affected by genetic variation during plant growth and development [3]. The majority of the identified leaf proteins from *Musa acuminate* Colla (AA) were found to be involved in energy metabolism. Moreover, the minority of leaf proteins identified by 2D-PAGE and MALDI-TOF MS were found to be involved in immunity and defense mechanisms [4]. In the case of leaves from normal banana and giant banana cultivar Prata Ana (AAB) with different genome from *Musa acuminate* Colla (AA) that were analyzed by 2D-PAGE and MALDI-TOF/TOF, the abundant proteins from the leaves of both normal banana and giant banana cultivar Prata Ana (AAB) were identified as being related to root metabolism, photosynthesis, protein translation carbon assimilation and nitrogen fixation [3].

In contrast, *M. acumimata* (BB group) "Kluai-Tanee" and *M. Xparadisiaca* (ABB group) "Kluai Nam Wa" with different genomes from *Musa acuminate* Colla (AA) and banana cultivar Prata Ana (AAB) have

not been reported in the protein reference map. In Thailand, *M. acumimata* BB (Kluai-Tanee) and *M. Xparadisiaca* ABB group (Kluai Nam Wa) are important types for leaf and fruit production. The analysis of protein compositions in banana leaves can increase the potential applications of these types of banana for both production efficiency and further improvement of banana through breeding. The aims of this study were to analyze and compare leaf proteins from different genomes of BB (Kluai-Tanee) and ABB (Kluai Nam Wa) by 2D-PAGE using known *Musa acuminate* Colla (banana) leaf proteins that were previously reported for comparison. The proteins from leaves of both banana types were compared, and two protein spots with different intensity between two banana types were selected for identification of each type by using LC-ESI-MS/MS method [5].

Materials and methods

Plant materials and field experiment

The banana varieties of both types used in this study were planted at the experimental farm of the Faculty of Agricultural Technology, Burapha University, Sakaeo Campus, Sakaeo, Thailand.

Protein extraction from leaves

Leaf samples were extracted by the method described previously [6] with some modifications. The leaf samples were ground into powder in a mortar with liquid nitrogen. The ground samples were suspended in lysis buffer (7 M urea, 2 M thiourea, 4 % (w/v) CHAPS, 2 % (w/v) DTT). After centrifugation at 12, 0000 rpm for 30 min at 4°C, the supernatant for each sample was collected and cleaned with the 2D-clean up kit to remove contaminants such as carbohydrate, lipids and others. (Amersham Bioscience, Sweden). The protein pellets were suspended in rehydration buffer (7 M urea, 2 M thiourea, 2 % (w/v) CHAPS, 2 mM DTT, 0.8 % (w/v) IPG buffer and 0.2 % bromophenol blue). The protein concentration was determined using the Bradford assay with bovine serum albumin (Amersham Biosciences, USA) as the standard.

2D-PAGE electrophoresis

Proteins were analyzed by 2D-PAGE as described by O'Farrel [7] and some modified method on linear (3-10) pH gradient. Protein samples of 100 µg each were solubilized in a focusing solution containing 7 M urea, 2 M thiourea, 0.3% DTT, 2% CHAPS and 2% IPG buffer corresponding to the pH gradient used. Isoelectric focusing (IEF) was conducted with a Ettan IPG phor 3 (Amersham Bioscience, USA) on 7 cm IPG strips using a gradient mode yielding 9,750 Vhr. After focalization, the gel strips were equilibrated for 15 min in buffer containing 50 mM Tris-HCl, pH 8.8, 6 M urea, 30% glycerol, 1% SDS and 25 mM DTT. A second 15 min equilibration step in the same solution containing 250 mM iodoacetamide instead of DTT was then performed. Proteins were then subjected to SDS-PAGE. IPG strip was placed on second dimension 15% SDS polyacrylamide gels. The gel was then stained with colloidal coomassie brilliant blue G250 solution. These gels were then washed with double distilled water until the background was clear. The gel images were captured using ImageQuant LAS 500 (GE Healthcare Bio-Sciences AB, Sweden) and protein spots were then analyzed by Image Master 2D Platinum v. 5.0 software (Amersham Biosciences, USA).

Protein Identification by LC-ESI-MS/MS

The protein spots with differential expressions were excised with trypsin using sequencing grade reagent (Promega, USA) according to the manufacturer's specifications. Each tryptic peptides were analyzed with a nano-liquid chromatography system (EASY-nLC II, Bruker) coupled to an ion trap mass spectrometer (Amazon Speed ETD, Bruker) equipped with an ESI nano-sprayer. The ESI-TRAP instrument was calibrated in the m/z range of 50-3000 using an internal calibration standard (Tune mix solution), which was supplied from Agilent. Bruker Daltonics software package, HyStar v.3.2, was used to control the ion trap device. LC–MS/MS spectra were analyzed using Compass Data Analysis v.4.0. Compound lists were exported as Mascot generic files (mgf) for further identification of proteins, which was performed by searching against the protein database from NCBIprot (Other green plants) using MASCOT MS/MS Ion Search program (www.matrixscience.com).

Results and discussion

In ours preliminary study, we separated Kluai Ta Nee and Kluai Nam Wa leaves proteins by 2D-PAGE using linear IPG strip (pH3-10) size 7 cm. Figure 1 showed the protein patterns of Kluai Ta Nee (Figure 1A) and Kluai Nam Wa (Figure 1B) leaves. The results demonstrated that approximately 50 and 44 protein spots were reproducibly detected for Kluai Ta Nee and Kluai Nam Wa leaves, respectively. The overall protein patterns of Kluai Ta Nee leaves were different from those of Kluai Nam Wa leaves. However, the molecular weights of major leaf proteins common to both Kluai Ta Nee and Kluai Nam Wa ranged from approximately 25 kDa to 70.0 kDa and pI (isoelectric point) range was between 4 and 7. The ranges of protein molecular weights and pI in previous study were wider than in our study. According to Lu et al.[4], the protein molecular weights of *M. acuminate* Colla (banana) leaves ranged from approximately 10 kDa to 100 kDa and the range of pI was from 3 to 10. However, the major leaf proteins from both normal and giant plants of *Musa* spp. cultivar Prata Ana (AAB) had molecular weights ranging from 14.0 kDa to 50.0 kDa and pI range was between 3 and 10 [3].

The protein patterns of two banana genomes were compared and analyzed. Some protein spots from each genome were separated and identified by Image Master 2D Trial. Table 1 summarized leaf proteins identified by pI and molecular weight on two referent gels compared with *M. acuminate* Colla (banana) leaf proteins reported previously [4]. The well-separated protein spots are known banana leaf proteins such as ribulose-1,5-bisphosphate carboxylase, oxygen-evolving enhancer protein and superoxide dismutase.

Table 2 summarized proteins identified by LC-ESI-MS/MS. Ribulose-1,5-bisphosphate carboxylase is an important primary enzyme in photosynthesis with a carboxylase that catalyzes the assimilation of carbon dioxide into organic compounds. Therefore, it is not surprising that we found ribulose-1,5-bisphosphate carboxylase in both Kluai Ta Nee and Kluai Nam Wa leaves, and these enzymes were also found in *M. acuminate* Colla leaf and *Musa* spp. cultivar Prata Ana (AAB) leaves [3, 4].

However, Kluai Nam Wa leaves had higher number of ribulose-1,5-bisphosphate carboxylase spots than Kluai Ta Nee leaves. In addition, ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit found in Kluai Ta Nee leaves had higher intensity than in Kluai Nam Wa leaves. Moreover, oxygen-evolving enhancer protein is one of protein complex of photosystem II found in thylakoid membranes [8]. We found that oxygen evolving enhancer protein from Kluai Nam Wa leaves had higher number of multiple spots than *M. acuminate* Colla leaves. Moreover, the authors found that a low quality protein: oxygen-evolving enhancer protein 1, chloroplastic-like, in Kluai Nam Wa leaves had higher intensity of protein bands than in Kluai Ta Nee leaves.

Interestingly, we found that superoxide dismutase from Kluai Ta Nee and Kluai Nam Wa leaves had multiple spots similar to that found in *M. acuminate* Colla leaves [4]. Superoxide dismutase acts as the first line of defense against ROS. It is induced by cold stress in plantain (*Musa paradisiaca* L.; ABB group) [2]. As the leaves of Kluai Ta Nee and Kluai Nam Wa were collected on 1st December, 2018, superoxide dismutase found in this study may respond to low temperature in the winter season. In addition, endochitinase was found in Kluai Ta Nee leaves, which was in agreement with that found in *M. acuminate* Colla (banana) leaves [4]. The general function of endochitinase is believed to be the hydrolysis of chitin in the cell walls of fungi and bacteria. Most of plant endochitinases are inducible with wounding, cold, pathogen infections, or hormones, like ethylene, methyl jasmonate and gibberellins [9]. In this study, four proteins (ribulose-1,5-bisphosphate carboxylase, oxygen-evolving enhancer protein superoxide dismutase and endochitinase) in Kluai Ta Nee and Kluai Nam Wa leaves had multiple spots. The differentiations of pI and/or molecular weight of these proteins are the results of post–translational modification [4].

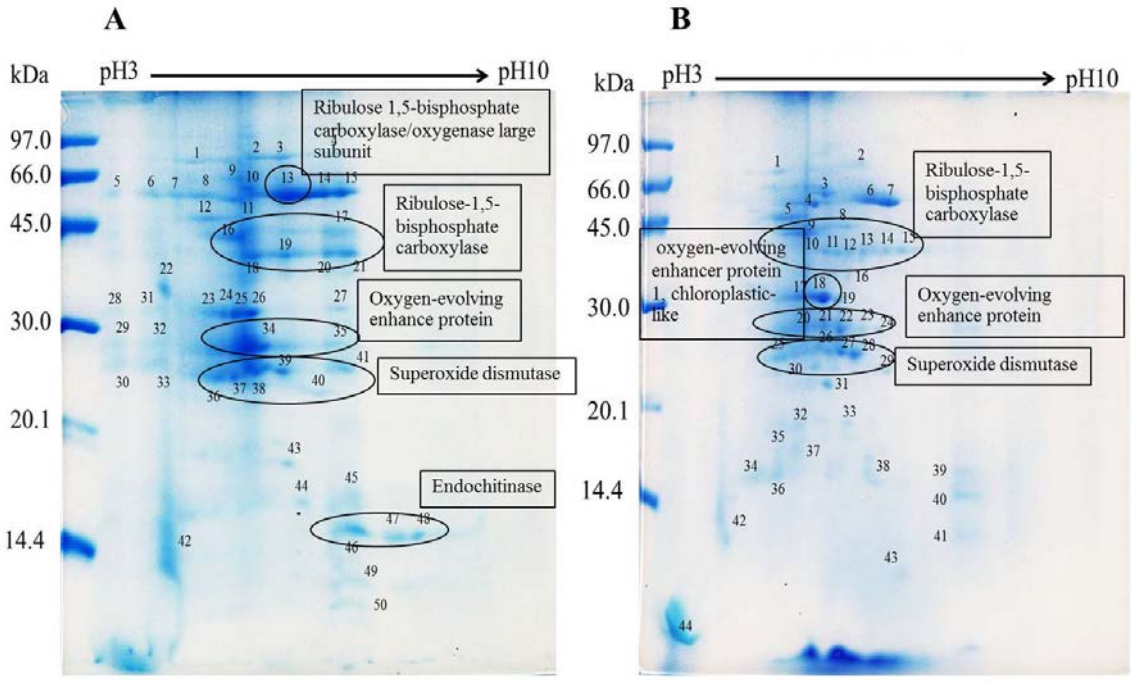

Figure 1 2D-PAGE of Kluai Ta Nee (A) and Kluai Nam Wa (B) leaf proteins.

Table 1 Summary of proteins identified by pI and molecular weight.

Banana leaves protein	pI		Molecular weight (kDa)	
	Experimental	Theory	Experimental	Theory
Kluai Ta Nee				
- Ribulose-1,5-	5.09	6.40 [4]	43	44.778 [4]
bisphosphate	6.71		44	
carboxylase	5.40		40	
	5.90		40	
	6.54		40	
	6.90		40	
- Oxygen-evolving	5.26	8.26 [4]	28	27.775 [4]
enhance protein	6.63		28	
- Superoxide dismutase	5.12	7.1 [4]	25	25.823 [4]
	5.41		25	
	5.85		25	
- Endochitinase	6.94	6.07 [4]	15	14.295 [4]
	7.60		14	
	7.94		14	
Kluai Nam Wa				
- Ribulose-1,5-	5.82	6.40 [4]	44	44.778 [4]

bisphosphate	5.12		43	
carboxylase	5.48		40	
	5.67		39	
	5.85		40	
	6.13		40	
	6.47		40	
	6.72		40	
- Oxygen-evolving	5.14	8.26 [4]	28	27.775 [4]
enhance protein	5.45		28	
	5.70		28	
	6.06		27	
	6.38		27	
- Superoxide dismutase	5.48	7.1 [4]	25	25.823 [4]
	5.71		25	
	5.95		25	

Table 2 Identification of the selected proteins from leaves of Kluai Ta Nee and Kluai Nam Wa identified by LC-ESI-MS/MS.

Spot No.	Match to	Protein name	Score	Theory		Calculated		Sequence coverage (%)	Peptide sequences
				pI	Mw. (kDa)	pI	Mw. (kDa)		
Kluai Ta Nee									
13	YP_008854433.1	Ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit [Musa textilis]	522	6.23	54.68	5.91	57	17%	KDTDILAAFRV, RFLFCA EAIFKA, REMTLGFVDLLD ,REMTLGFVDLLR D, KDDE NVNSQ PFMRW, KTFQGP PHGIQVERD, KD DENVNSQPFMR W, KWSPELAAA CEVWKE, KGHY LNATAGTCEEM MKR
Kluai Nam Wa									
18	XP_009412420.1	LOW QUALITY PROTEIN: oxygen-evolving enhancer	508	6.77	36.23	5.43	31	22%	KRLTYDEIQSKT, KRLTY DEIQSKT, KRLTYDEIQ SKT,

	protein 1, chloroplastic-like [Musa acuminata subsp. malaccensis]	KRLTYDEIQSKT, RLTYDEIQSKT, KDGIDYAAVTV QLPGGERV, KD GIDYAAVTVQLP GGERV, KDGIDY AAVTVQLPG GE RV, RVPFLFTIKQ, RGGSTGYDNAV ALPAGGRG, RGD EEELSKENIKN, KIQGVWYAQLEQ

Conclusions

The 2D-PAGE patterns of Kluai Ta Nee leaves revealed ribulose-1,5-bisphosphate carboxylase, oxygen-evolving enhancer protein and superoxide dismutase with different pI and number of isoforms compared with the 2D-PAGE patterns of Kluai Nam Wa leaves. Moreover, 3 isoforms of endochitinase were found in Kluai Ta Nee leaves only. The major protein, ribulose 1,5- bisphosphate carboxylase/oxygenase large subunit found in Kluai Ta Nee leaves had higher intensity than in Kluai Nam Wa leaves. However, a low quality protein: oxygen-evolving enhancer protein 1, chloroplastic-like found in Kluai Nam Wa leaves had higher intensity than in Kluai Ta Nee leaves. The polymorphism of these proteins found in Kluai Ta Nee leaves and Kluai Nam Wa leaves might be responsible for different biological functions in each type. The further investigations are required to use other techniques for identification, characterization and better understanding on functional properties of the proteins.

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