Genome-wide Identification and Characterization of the Pectin Methylesterase (PME) and Pectin Methylesterase Inhibitor (PMEI) Gene Family in the Banana A-genome (Musa acuminata) and B-genome (Musa balbisiana)

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Abstract

Pectin methylesterase (PME) is a ubiquitous cell-wall-associated enzyme that catalyzes the demethylesterification of homogalacturonan in the cell wall. The activity is regulated in part by pectin methylesterase inhibitor (PMEI), which can bind to the active site of PME. This mechanism may result in either rigidification or softening of the cell wall depending on the mechanism of demethylesterification. This research aims to characterize the structure and function of the PME and PMEI gene family in A (Musa acuminata) and B (Musa balbisiana) banana genomes. We annotated based on domain PME (PF019095) and PMEI (PF04043) using the PFAM database. We analyzed the size, weight, isoelectric point of the protein, signal peptide, transmembrane domain, subcellular localization of proteins, prediction cleavage motif and position, phylogenetic analysis and conserved residues.

As a result, we found great diversity of PME in the A genome (70 PME: 41 PME type 1 and 29 PME type 2; 23 PMEI) and B-genome (60 PME: 38 PME type 1,22 PME type 2; 23 PMEI) within the parameters. Phylogenetic analysis classified the PME into six monophyletic groups. The gene family of PME and PMEI in banana genomes had a wide variety of patterns of structural, biochemical and functional characteristics.

Keywords: Pectin methylesterase, pectin methylesterase inhibitor, A genome, B genome, gene family.

Introduction

Plant and animal cells differ in the presence of their cell walls position to the plasma membrane. The cell wall consists of several components including pectin which is a complex polysaccharide found in the primary cell wall and modified by the pectin methylesterase (PME) enzyme associated with the cell wall. Specifically, PME catalyzes the homogalacturonan demethylesterification process (the most abundant polymer of pectin) on the cell wall. Its activity is partially regulated by pectin methylesterase inhibitors (PMEI) which can bind to the active side of PME and produce a 1:1 complex. The results of this activity can make the cell wall stiff or soft depending on the demethylesterification mechanism[11,20].

PME plays a role in critical physiological processes in plants such as microsporogenesis, pollen growth, seed germination, root development, stem extension, fruit maturation and response to pathogens[29]. Structurally, PME classified as PME type 1 which has two domains (PME and PMEI) that are separated by one or two binding motifs that will divide when entering the cell wall. PME type 2 only has a PME domain and PME Inhibitor (PMEI). PME and PMEI are initiated by signal peptides or transmembrane domains[38]. PME isoforms of a gene family are distinguished by molecular weight, isoelectric points and biochemical activities that are constitutively expressed or regulated in specific tissues at growth and developmental stages. The function of PME and PMEI is maintained by conserved residues for protein stability. PME had 11 conserved residues and PMEI had 33 sustainable residues[11].

Most of the research on PME and PMEI focuses on dicotyledonous plants such as *Arabidopsis thaliana* in the development of silique development[26], *Aspergillus niger*[12,25], willow[16], orange[8], pea[35], maize[36], mung bean[5], grape ripening (*Vitis vinifera*)[4], tomatoes[15,34] and flax[1,32]. However, research on monocot plants is still limited especially regarding its structural and functional characterization.

In this study, we aimed to characterize PME and PMEI gene families of banana as it is an essential commodity in the world and Indonesia[2,3]. Bananas have four genome types: *Musa acuminata* (A Genome), *Musa balbisiana* (B Genome), *Musa schizocarpa* (S Genome) and the *Australimusa* species (T Genome)[17]. In Indonesia, the highest diversity of bananas comes from the combination of genomes A and B[17]. Currently, A and B genomes have been sequenced[9,10,13,28]. We used A and B genomes as material for the structural and functional characterization on PME and PMEI gene families.

Material and Methods

Banana A and B genomes sequences: The version 2 of genome A (*M. acuminata*)[28] and the draft version of genome

B (*M. balbisiana*) sequences were obtained from the Banana Genome Hub[13]. We used the protein-coding sequence (CDS) and nucleotides.

Annotation of the PME and PMEI domain on banana A and B genomes: We annotated the CDS based on thr PME domain (PF019095) and PMEI (PF04043) from PFAM 30.0 database[33]. PME type 1 consist of two domains, the PMEI (PF04043) as the pro-region domain and PME domain (PF019095). PME type 2 only can have the PME domain and PMEI only has the PMEI domain.

Length, weight and isoelectric point (pI) of the protein: Protein sequences from both genomes were characterized based on the isoelectric point, weight and length using the Vector NTI, version 11.524. The peptide signal and cleavage position part were previously removed.

Signal peptides, transmembrane domains and subcellular localization of proteins: We used SignalP 4.1[31], TMHMM v.2.0[22], WoLF PSORT[19], Plant-mPloc[7] servers to predict the presence of signal peptides, the existence of the transmembrane domain and subcellular localization respectively. All websites were accessed on October 13th, 2016.

Prediction of the cleavage site position and motive in PME type 1: The proteolytic cleavage site was predicted using the pattern of protease recognition[30,38]. We searched Protein Pattern Find (http://www.bioinformatics.org/sms2/protein_pattern.html) using the motif pattern of [RQK] [RKEHLN] [LDMI] [LMAKR].

Sustainable residue (conserved residue) prediction: The presence of protein residues is important for PME and PMEI protein activity[11]. PME sequence had 11 important sustainable residues consisting of six active sites/substrate bindings (F80, Y135, FI56, Y218, W223, W248), three catalytic residues (D132, D153, R221) and two protein stabilizers (Q109, Q131). The PMEI sequence had 33 sustainable residues including residues that interact with the active sites of PME (T73, E76, N77, T113, D116), residues that form disulfide bridges (C9, C18, C74, C114) and some other residues that function to maintain the structure of these proteins such as non-polar bundle hairpin interface residues (I5, I8, L17, A21, L22, K31, L33, L36, L138, L141, V144, I145, L148), polar bundle interfaces (N14, E23, R27, D32, D137) and acidic patches on the α2 helix (E76, D80, D83) and α3 helix (D96, D109, D116).

We aligned the PME and PMEI protein sequences from the A and B genomes to the structural template sequence from tomatoes (*Solanum lycopersicum*; PME1_SOLLC, SwissProt P14280) and kiwi (*Actinidia deliciosa*; PMEI_ACTDE, SwissProt P83326) using ClustalW in MEGA 7 to identify the sustainable residues.

Molecular phylogenetic analysis: We determined the evolutionary relationship between A (*M. acuminata*) and B (*M. balbisiana*) banana genomes with other species such as flax (*Linum usitatissimum*), cassava (*Manihot esculenta*), *Ricinus communis*, black cottonwood (*Populus trichocarpa*) and *Arabidopsis thaliana*. We obtained the sequences from the Phytozome v.11.0 server (https://phytozome.jgi.doe.gov/pz/portal.html) using the PFAM accession numbers PF01095 (PME) and PF04043 (PMEI). We aligned sequences using MUSCLE14 in MEGA 7 23, then bootstrapped for 10,000 with the maximum-likelihood algorithm from IQ-TREE. The phylogenetic tree was visualized using FigTree v1.4.2.

Results and Discussion

PME and PMEI domain annotation on the banana Genome A and B: 70 PME (41 PME type 1 and 29 of type 2) and 23 PMEI were found in genome A and 60 PME and 23 PMEI in genome B. PME type 1 encodes domain PF01095 and PMEI (PF04043) while PME type 2 only encodes domain PF01095. These results were used for further analysis (Tables 1 to 6).

Length, weight and isoelectric point (pI) of the mature protein: The results of the analysis of the mature length of type 1 PME protein and PME type 2 from both genomes are in the range of 48–1,014 amino acids (AA), whereas PMEI of both genomes is in the range of 166–299 AA (Tables 1 to 6). This long-range of amino acids is the same as the long-range in flax31.

The mature molecular weight of PME type 1 protein in both genomes is in the range of 36.3 to 107.4 kDa, PME type 2 protein ranged from 5.4 to 113 kDa and PMEI ranged from 17.8 to 28.4 kDa. The molecular weight of bananas PME was also in the same weight range as dicotyl plants[18,31]. The isoelectric point (pI) is one of the factors influencing the PME function. These isoelectric points affect the demethylesterification mechanism; an acidic pI will result in random demethylesterification to soft cell walls and basic pI makes the cell walls rigid[37]. The isoelectric point in both genomes is mostly alkaline, but in the PME type 1 in genome B, the pI was equally between the acid and base pI (Table 1–4). Compared to dicotyl plants, the pI of PMEI flax is mostly acidic[32].

Cleavage site in the PME type 1: PME type 1, before becoming mature, will proteolytically divide on the cleavage side. Cleavage-binding motifs can be on either side, but the cleavage side before leaving the Golgi body is only on one side[38]. Based on the number of binding motifs in the two genomes (Figure 1), the highest number has a binding motif on two sides of the cleavage (20 of 41 proteins in the A genome and 20 of 39 proteins in the B genome). In both genomes, the binding motifs in the two cleavage positions are separated by 14–30 residues which are nearly the same as the range of residues of the flax plants 14–33[32].

Table 1
PME type 1 Characteristics from A Genome

S.N.	PME Annotation	Protein Characteristics			Binding Motif			Subcellular Localisation				Conserved Residues		
		AA	Kda	pI	BM1	BM2	Position	SP	TM	WolF PSORT	Plant-mPloc	AS	ST	CR
1	*MaPME3*	556	61.4	6.17	RKLL	RRLL	213–229	-	+	v, ch, n, m, ex, pe	cw	5	2	3
2	*MaPME6*	596	65.5	8.8	RKLL	RRLL	253–273	-	+	n, ch, cy, pl, v, er, pe	cw	6	2	3
3	*MaPME7*	506	54.2	5.15	RRLL	RRLL	169-183	+	+	ch, ch_m, m	cw	6	2	3
4	*MaPME8*	548	58.8	5.28	-	RRLL	225	+	-	ex, ch, m, v, er	cw	5	2	3
5	*MaPME9*	550	59.7	8.06	RNLL	-	221	+	+	ch	cw	5	2	3
6	*MaPME10*	578	62.9	6.15	-	RKLL	239	-	+	cy, pl, er, n, ch, v	cw	5	2	2
7	*MaPME13*	527	58.5	9.23	RRLL	-	206	+	-	v, ch, g, n	cw	4	2	2
8	*MaPME15*	543	59	9.1	RRLM	RKLL	205–227	+	+	ch, v, m, ex	cw	6	2	3
9	*MaPME17*	553	59.3	7.73	-	RKLL	231	-	+	ch, m, pl	cw	6	2	3
10	*MaPME18*	558	59.6	6.41	-	-	-	+	-	ch	cw	5	2	3
11	*MaPME20*	525	58.7	9.84	RRLL	-	204	+	+	ex, ch, v, n, cy, m, pl	cw	4	2	3
12	*MaPME21*	559	61.9	6.79	RKLL	RRLL	213–229	+	+	ex, n, er, g, ch, m, pl	cw	5	2	3
13	*MaPME23*	352	38	9.7	RELL	RRLL	220–242	+	+	ch, cy	cw	1	0	0
14	*MaPME25*	533	57	8.59	RRLM	RKLL	197–211	+	-	ch	cw	5	2	3
15	*MaPME26*	639	70.9	9.4	RRLL	RRML	285–315	-	+	n, er, cy, v, ch, m	cw	6	2	3
16	*MaPME32*	560	59.6	5.67	-	-		+	+	ch, m, pl	cw	5	2	3
17	*MaPME33*	567	62	8.63	RRLL	RKLL	215–236	+	+	v, ex, ch, g, pl	cw	6	2	3
18	*MaPME34*	568	61.5	8.67	-	RRLR	190	-	+	m, ch, cy_m, cy, pl	cw	6	2	3
19	*MaPME35*	522	56.3	8.7	RRLL	RKLL	183–199	+	-	ex, ch, er, pe	cw	6	2	3
20	*MaPME36*	522	57.9	8.37	RRLL	-	182	+	-	v, ch, ex, er, g	cw	5	2	3
21	*MaPME37*	549	59.2	7.1	RRLM	RRLL	213–227	+	-	ch	cw	5	2	3
22	*MaPME39*	578	63.6	6.32	KHLM	-	312	-	-	ch, v, er, pe, n, cy, pl	cw	6	2	3
23	*MaPME44*	540	60.4	8.34	RKMR	-	194	+	-	er, er_pl, n, pl, ch, cy, m	cw	6	2	3
24	*MaPME45*	564	60	8.84	RKLL	RRLL	227–243	-	+	er, er_pl, n, m, ch	cw	5	2	3
25	*MaPME46*	549	58.8	8.62	RELA	-	105	+	+	ch, m	cw	5	2	3
26	*MaPME47*	550	58.9	8.62	RELA	-	105	+	+	ch, m	cw	5	2	3
27	*MaPME48*	489	52.8	7.11	RRLL	RKLL	223–249	-	-	ch, n, cy, m, pl, er, ct	cw	2	0	1
28	*MaPME49*	592	64.9	5.74	RRLL	RRLL	241–267	-	+	er, er_pl, n, v, cy, pl	cw	5	2	3
29	*MaPME55*	529	57	5.3	-	RRLL	205	-	-	ch	cw	5	2	3
30	*MaPME56*	594	65.1	8.57	RRLL	-	245	-	+	n, cy, cy_pe, v, er	cw	6	2	3
31	*MaPME57*	632	68.1	9.1	RKLL	RRLL	294–310	-	+	ch, n, cy, er, m, v	cw	5	2	3
32	*MaPME58*	557	61	8.57	RRLL	RKLL	216–234	+	+	v, g, ex, ch, cy, pl	cw	6	2	3
33	*MaPME59*	594	64.8	7.32	RRLL	RRIL	244–270	-	+	n, cy, cy_pe, v, er	cw	6	2	3
34	*MaPME60*	557	59.6	5.72	-	-		+	-	ch, n, m	cw	5	2	3
35	*MaPME61*	591	64.9	8.99	RKLL	RRLL	247–268	-	+	ch, pl, n, cy, v	cw	6	2	3
36	*MaPME63*	568	62.9	8.01	-	RRLL	243	+	-	ch, er, n, m, ex	cw	6	2	3
37	*MaPME64*	566	62.1	7.04	-	RRLL	244	-	+	ch, m	cw	6	2	3
38	*MaPME66*	567	61.5	8.85	-	RKLL	245	-	+	ch, m	cw	6	2	3
39	*MaPME68*	573	62.1	6.77	RRLL	-	248	+	+	ch, ex, n, cy, pl, v	cw	6	2	3
40	*MaPME69*	559	61.9	6.79	RKLL	RRLL	213–229	-	+	v, ch, g, n, m	cw	5	2	3
41	*MaPME70*	576	62.5	8.49	RRLL	RKLL	233–255	-	+	ch, n, cy, pl	cw	6	2	3

SP: Signal Peptides; TM: Transmembrane Domain. Subcellular Localisation: ch: chloroplast; cw: cell wall; cy: cytosol; er: endoplasm reticulum; ex: extracellular/cell wall; g: golgi; m: mitochondria; n: nucleus; pl: plasma membrane; v: vacuola; pe: peroxisome; ct: cytoskeleton. (AS): Active Sites (max. 6); (ST): Stabilizer Residues (max. 2). CR: Catalytic Residues (max. 6)

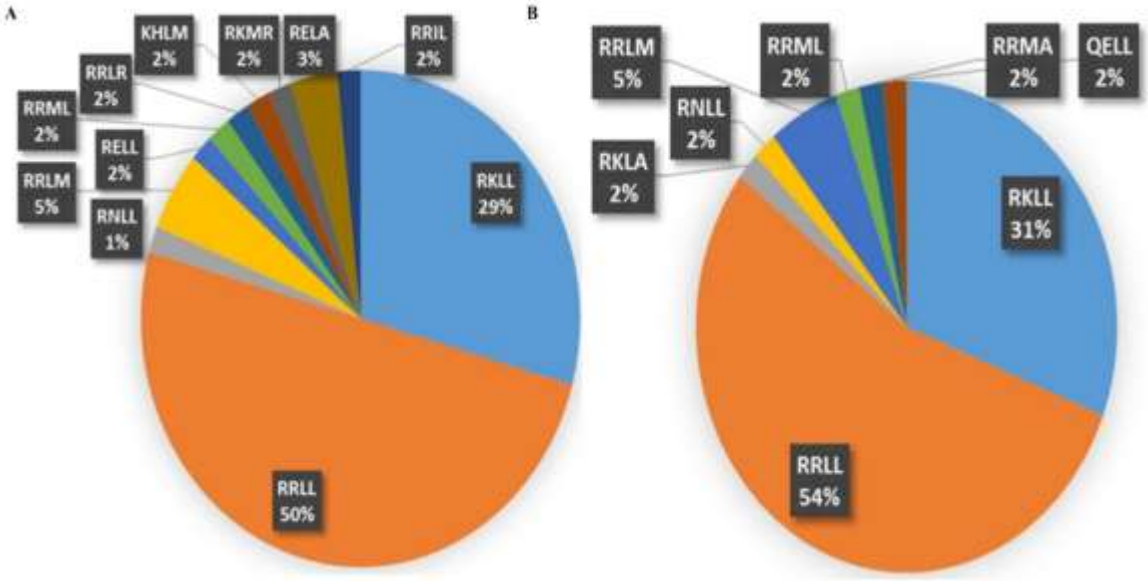

Figure 1: Binding Motif in Banana Genomes (A: A Genome, B: B Genome)

Table 2
PME type 2 Characteristics from A Genome

S.N.	PME	Protein Characteristics			Subcellular Localisation				Residu Lestari		
		AA	Kda	pI	SP	TM	WolF PSORT	Plant-mPloc	AS	ST	CR
1	*MaPME1*	379	41.6	9.13	+	-	ch, v, n, cy_n, m, ex	cw	6	2	3
2	*MaPME2*	331	36.8	6.59	+	+	ch, v, g, n	cw	4	1	3
3	*MaPME4*	447	49.7	8.05	+	+	v, ch, n, m	cw	4	2	3
4	*MaPME5*	339	37.7	8.51	+	+	v, ch, ex, er,n, cy, m	cw	3	1	2
5	*MaPME11*	351	38	8.58	-	-	cy, ch, n	cw	3	2	2
6	*MaPME12*	230	24.1	5.23	-	-	n, m	cw	1	2	1
7	*MaPME14*	344	38.3	8.05	+	-	er, ch, ex, pl, cy	cw	3	2	3
8	*MaPME16*	347	38.7	8.08	+	-	ex, cy, ch	cw	3	2	3
9	*MaPME19*	274	31.2	7.76	+	+	ch, ex, n, cy	cw	4	2	3
10	*MaPME22*	161	17.7	6.91	-	-	ch, n	cw	1	2	1
11	*MaPME24*	394	43.2	9.49	-	-	cy, ch, cy_n, m, n	cw	2	2	3
12	*MaPME27*	404	43.7	5.83	+	+	v, ch, ex, g, pl	cw	3	2	3
13	*MaPME28*	377	41.5	9.01	+	-	ch, v	cw	6	2	3
14	*MaPME29*	342	37.6	9.22	+	-	ch, v, cy	cw	6	2	3
15	*MaPME30*	404	44.6	8.91	+	-	ch, v	cw	6	2	3
16	*MaPME31*	373	40.4	6	-	+	cy, er, er_pl, n, m	cw	2	1	3
17	*MaPME38*	343	38.2	5.92	-	-	cy, cy_n, ch, n, m	cw	5	2	3
18	*MaPME40*	326	36.1	5.18	+	-	ch, ex	cw	4	1	3
19	*MaPME41*	197	21.8	7.01	+	-	ch, n, cy	cw	1	1	2
20	*MaPME42*	313	34.9	9.29	+	-	ch, ex	cw	4	1	3
21	*MaPME43*	331	36.8	7.71	+	+	ch, v, g, n	cw	4	1	3
22	*MaPME50*	348	37.9	5.92	+	-	ch, v, ex, er	cw	4	2	3
23	*MaPME51*	389	43.1	9.29	+	-	ch, cy	cw	6	2	3
24	*MaPME52*	350	38.6	9.04	+	-	ch	cw	6	2	3
25	*MaPME53*	388	43	9.2	+	-	ch, cy	cw	6	2	3
26	*MaPME54*	48	5.4	4.63	-	-	cy, n, ch	cw	1	2	1
27	*MaPME62*	194	21.2	8.4	+	+	ch, v, ex, er	cw	0	2	1
28	*MaPME65*	317	35.6	7.22	-	-	cy, n, ch	cw	6	2	3
29	*MaPME67*	335	37.6	8.84	+	+	v, er, ch, g, n	cw	4	2	3

SP: Signal Peptides; TM: Transmembrane Domain. Subcellular Localisation: ch: chloroplast; cw: cell wall; cy: cytosol; er: endoplasm reticulum; ex: extracellular/cell wall; g: golgi; m: mitochondria; n: nucleus; pl: plasma membrane; v: vacuola; pe: peroxisome; ct: cytoskeleton. (AS): Active Sites (max. 6); (ST): Stabilizer Residues (max. 2). CR: Catalytic Residues (max. 6).

The most common motifs in both genomes are RRLL and RKLL. The common motifs of the two genomes are RRLM, RRML and RNLL. Different motifs between the two genomes including those in the A genome are motifs of RELL, RRLR and KHLM, while in the genome B there are RKLA, RRMA and QELL (Table 1 and table 4).

The monocotyledons binder motif is similar to the dicotyledonous motifs such as RKLL, RRLL, RRML and RELL. Additionally, there are motifs in dicotyledonous plants but not in monocotyledons such as RRVL, RKVA, RRLW, RKLR, RRKL, RKLK, RKVL, GRLL, REYL and RRFL32.

Subcellular localization: Signal peptides and transmembrane domains function as a signpost of proteins to the cell wall and prevent premature proteins from PME and PMEI before reaching the cell wall. Subcellular localization of PME is certainly in the cell wall and PMEI is generally in the plasma membrane[38]. In A genome, there are 21 PME and 9 PMEI that have signal peptides and transmembrane domains, while in the B genome, there are 17 PME and 8 PMEI. According to the mPLoc result, PMEs in both genomes are located on the cell wall, while PMEI is located on the cell membrane. We define the number of PME and PMEI based on the parameters of the signal peptides, transmembrane domains and predictions of the location of PME on cell walls and PMEI on plasma membranes using two WolF PSORT and mPLoc (Figure 2).

In the A genome, 12 PME were identified which only have signal peptides and are located on the cell wall according to mPLoc, while 7 PMEIs have signal peptides and are located in the plasma membrane according to PSORT and mPLoc. Similarly, in the B genome, 16 PME were observed using the signal peptides and the transmembrane domains were also located in the cell wall.

Molecular phylogenetic analysis: The monocot and dicotyledonous species were separated and defined as six monophyletic groups (Figure 3). A group consists of the PME type 1 domain, which has a cleavage site and signal peptide but does not have a transmembrane domain.

Table 3
PMEI Characteristics from A genome

S.N.	PMEI	Protein Characteristics			Subcellular Localisation				Conserved Residues					
		AA	Kda	pI	SP	TM	WolF PSORT	Plant-mPloc	*	§	□	£2	£3	¥
1	*MaPMEI1*	199	21.2	8.66	+	-	ch, cy, m	cm	8	1	4	2	1	4
2	*MaPMEI2*	214	23	8.81	+	+	ch, n, ex	cm	6	0	4	3	2	4
3	*MaPMEI3*	200	21.5	5.82	+	-	ex, v	cm	6	0	4	3	1	4
4	*MaPMEI4*	194	21	8.82	+	+	ch, m	cm	7	1	4	3	1	4
5	*MaPMEI5*	181	18.7	8.42	+	-	ch	cm	10	0	2	2	1	4
6	*MaPMEI6*	178	18.1	4.66	+	+	ex, v	cm	9	0	3	0	1	4
7	*MaPMEI7*	196	21.1	9.89	+	-	ch, ex, v	cm	7	1	4	3	1	4
8	*MaPMEI8*	205	21.4	6.14	+	+	ch, ex	cm	8	1	3	2	1	4
9	*MaPMEI9*	217	23.7	8.85	+	+	ch, v	cm	6	0	4	2	1	4
10	*MaPMEI10*	194	21.1	10.39	+	+	ch, v, ex	cm	7	0	3	2	1	4
11	*MaPMEI11*	209	22	6.46	+	-	ch, m	cm	7	0	4	3	1	4
12	*MaPMEI12*	299	31.2	5.59	+	-	ch	**cm**	10	1	3	3	2	4
13	*MaPMEI13*	206	22.1	8.67	+	-	ex, ch, cy	cm	6	0	4	3	2	4
14	*MaPMEI14*	277	28.4	5.24	+	-	ch. cy	**cm**	9	0	4	3	2	4
15	*MaPMEI15*	194	21	10.34	+	+	ch, cy, m	cm	8	0	4	3	1	4
16	*MaPMEI16*	224	23.8	5.92	-	+	ch, m, er, n, pl	cm	10	1	3	2	2	4
17	*MaPMEI17*	183	19.2	9.49	+	-	ch	cm	6	0	0	1	0	4
18	*MaPMEI18*	198	21.2	8.65	+	+	ex, xh	cm	7	0	4	3	1	4
19	*MaPMEI19*	185	19	7.07	+	-	ex, v, ch, er	cm	9	0	4	2	1	4
20	*MaPMEI20*	187	19.6	6.35	+	-	ex, ch, n	cm	11	1	2	2	2	4
21	*MaPMEI21*	201	21.5	7.76	+	+	ex, xh, m	cm	6	0	3	3	2	4
22	*MaPMEI22*	194	21	11.27	+	-	ch, ex, xy, m	cm	7	1	4	3	1	4
23	*MaPMEI23*	198	21	7.78	+	-	ex, v, cy	cm	7	1	3	2	1	4

SP: Signal Peptides; TM: Transmembrane Domain. Subcellular Localisation: ch: chloroplast; cw: cell wall; cy: cytosol; er: endoplasm reticulum; ex: extracellular/cell wall; g: golgi; m: mitochondria; n: nucleus; pl: plasma membrane; v: vacuola; pe: peroxisome; ct: cytoskeleton. Conserved residues as follows: (*) non polar bundle-hairpin interface (max. 12); (§) polar bundle-hairpin interface (max. 6); (□) polar residues interact with PME aromatic residues (max. 5); (£2) acidic patch on α2 helix (max. 3); (£3) acidic patch on α3 helix (max. 3); (¥) cysteine for disulfide bridge (max. 4).

Table 4
PME type 1 Characteristics from B Genome

S. N.	PME	Protein Characteristics			Binding Motif			Subcellular Localisation				Conserved Residues		
		AA	Kda	pI	BM1	BM2	Position	SP	TM	WolF PSORT	Plant-mPloc	AS	ST	CR
1	*MbPME4*	556	61.3	6.61	RKLL	RRLL	213–229	-	+	v, ch, g, n, m	cw	5	2	3
2	*MbPME7*	635	69.6	8.74	RKLL	RRLL	317–337	-	+	ch, er, n, ct_m, cy	cw	5	2	3
3	*MbPME8*	506	54.3	4.95	RRLL	RRLL	169–183	+	-	ch, m, n	cw	6	2	3
4	*MbPME9*	548	58.8	5.05	RKLA		139	+	-	ex, ch, m, er, g	cw	5	2	3
5	*MbPME10*	550	59.6	6.45	RNLL		221	+	-	ch	cw	5	2	3
6	*MbPME11*	579	63.1	5.87		RKLL	242	-	+	cy, pl, er, v	cw	5	2	2
7	*MbPME13*	780	84.7	8.34	RRLM	RKLL	205–236	+	+	pl, g, v	ch	6	2	3
8	*MbPME15*	553	59.6	7.72		RKLL	231	-	+	ch, n	cw	6	2	3
9	*MbPME16*	558	59.5	6.35				+	-	ch	cw	5	2	3
10	*MbPME18*	525	58.6	9.88	RRLL		204	+	+	ch, v, n, cy, m, pl	cw	4	2	3
11	*MbPME19*	559	62	7.66	RKLL	RRLL	213–229	+	+	ex, v, g, ch, n, m, pl	cw	5	2	3
12	*MbPME20*	334	36.3	9.63		RRLL	236	-	+	ch, cy	Cw	1	0	0
13	*MbPME22*	533	57.2	9.05	RRLM	RRLL	197–211	+	-	ch, n	cw	5	2	3
14	*MbPME23*	585	64.8	9.35	RRLL	RRML	232–262	-	+	n, er, cy, pl	cw	6	2	3
15	*MbPME24*	589	64.5	9.17	RKLL	RRLL	245–266	-	+	ch, pl, er, cy, m, v	cw	6	2	3
16	*MbPME28*	542	57.4	5.46				+	-	ch, v, ex, n, m	cw	5	2	3
17	*MbPME29*	559	61.2	8.41	RRLL	RKLL	215–236	+	+	v, ex, ch, g, pl	cw	6	2	3
18	*MbPME30*	363	39.4	8.18	RRLL	RKLL	204–225	+	+	ex, ch, m, v, er	cw	3	0	1
19	*MbPME31*	647	70.4	9.2		RRMA	231	+	+	ch	cw	6	2	3
20	*MbPME32*	534	57.6	5.96	RRLM	RRLL	198–212	+	-	ch, n, pl, ex	cw	5	2	3
21	*MbPME34*	571	62.9	6.39	RRLL		239	+	+	n, er, er_pl, pl, ch, m	cw	6	2	3
22	*MbPME39*	539	60.3	8.33		QELL	225	+	-	n, er, er_pl, pl, ch, cy	cw	6	2	3
23	*MbPME40*	598	63.3	8.66	RKLL	RRLL	246–262	-	+	n, er, er_pl, pl, m, v	cw	5	2	3
24	*MbPME41*	999	107.4	8.66	RRLL	RRLL	720–746	+	-	ch, m	cw	5	2	3
25	*MbPME42*	592	65.1	5.93	RRLL	RRLL	241–267	-	+	n, er, cy, v, cy_pe	cw	5	2	3
26	*MbPME45*	543	58.3	4.93		RRLL	219	+	-	ch, m, v	cw	4	2	3
27	*MbPME46*	594	65	8.78		RRLL	245	-	+	n, cy, cy_pe,v, er	cw	6	2	3
28	*MbPME47*	580	61.9	8.93	RKLL	RRLL	242–258	-	+	n, ch, er, cy	cw	5	2	3
29	*MbPME48*	569	62.5	8.4	RRLL	RKLL	216–234	+	+	v, ex, g, ch, pl	cw	6	2	3
30	*MbPME49*	573	61.3	5.29				-	-	n, m, cy	cw	5	2	3
31	*MbPME50*	591	64.9	9.05	RKLL	RRLL	247–268	-	+	ch, n, pl, er, cy	cw	6	2	3
32	*MbPME52*	568	62.9	6.9		RRLL	243	+	-	ch, er, n, ex	cw	6	2	3
33	*MbPME53*	566	62.2	7.02		RRLL	244	-	+	ch, m	cw	6	2	3
34	*MbPME54*	617	67.1	8.92		RKLL	245	-	+	ch, m, n	cw	6	2	3
35	*MbPME56*	573	62.2	7.68	RRLL		248	+	+	ch, ex, n, cy, pl, v	cw	6	2	3
36	*MbPME57*	559	61.8	6.52	RKLL	RRLL	213–229	-	+	ch, v, g, n	cw	5	2	3
37	*MbPME58*	576	62.4	8.59	RRLL	RKLL	233–255	-	+	ch, n, cy	cw	6	2	3
38	*MbPME60*	522	56.3	8.8	RRLL	RKLL	183–199	+	-	ex, ch, cy	cw	6	2	3

SP: Signal Peptides; TM: Transmembrane Domain. Subcellular Localisation: ch: chloroplast; cw: cell wall; cy: cytosol; er: endoplasm reticulum; ex: extracellular/cell wall; g: golgi; m: mitochondria; n: nucleus; pl: plasma membrane; v: vacuola; pe: peroxisome; ct: cytoskeleton. (AS): Active Sites (max. 6); (ST): Stabilizer Residues (max. 2). CR: Catalytic Residues (max. 6)

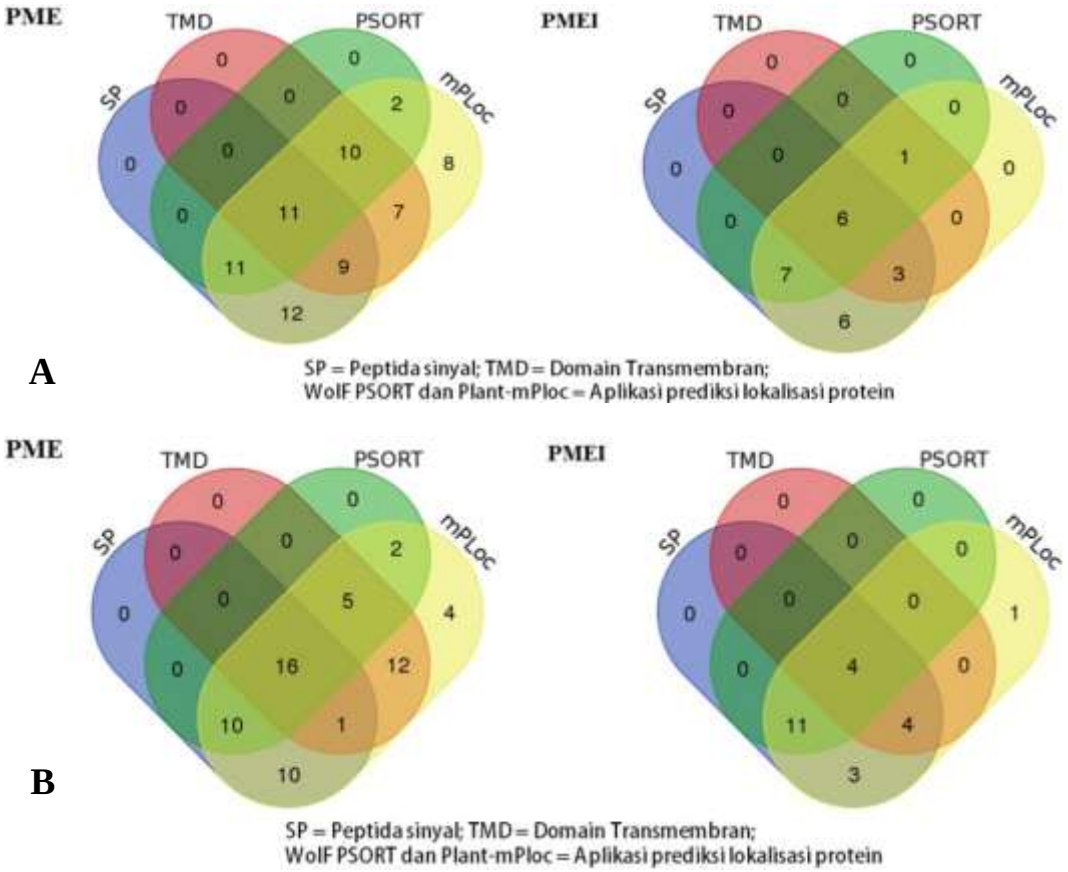

Figure 2: PME and PMEI in numbers for Signal Peptide (SP), Transmembrane Domain (TMD), Subcelullar Localisation using Wolf PSORT and Plant m-Ploc in cell wall and plasma membrane (A: A Genome, B: B Genome).

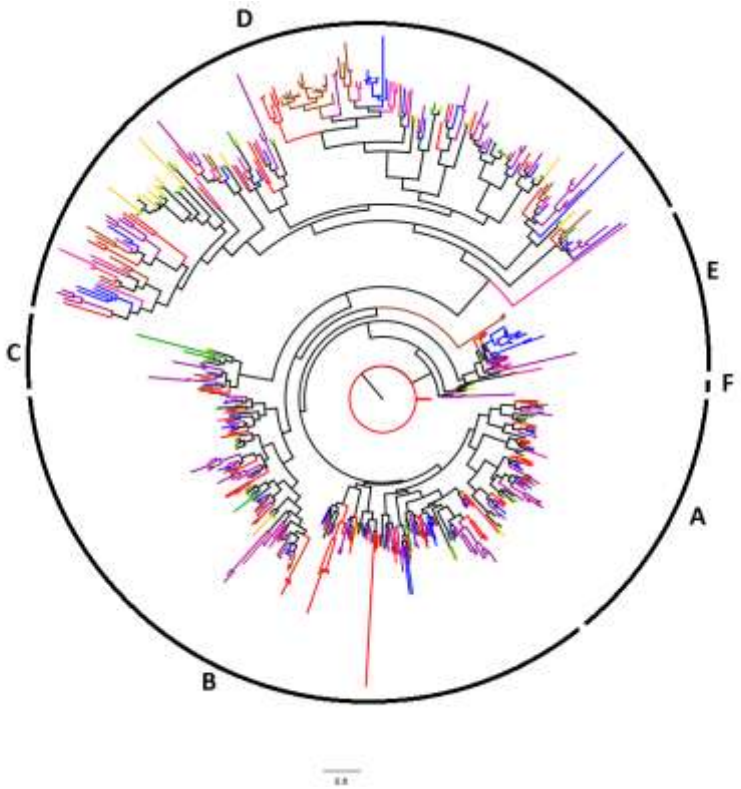

Figure 3: Phylogenetic Tree of PME. Violet: *Linum usitatissimum*; Brown: *Manihot esculenta*; Pink: *Ricinus communis*; Blue*: Populus trichocarpa*; Red*: Arabidopsis thaliana*; Green: *Musa acuminata* (A Genome); Yellow: *Musa balbisiana* (B Genome)

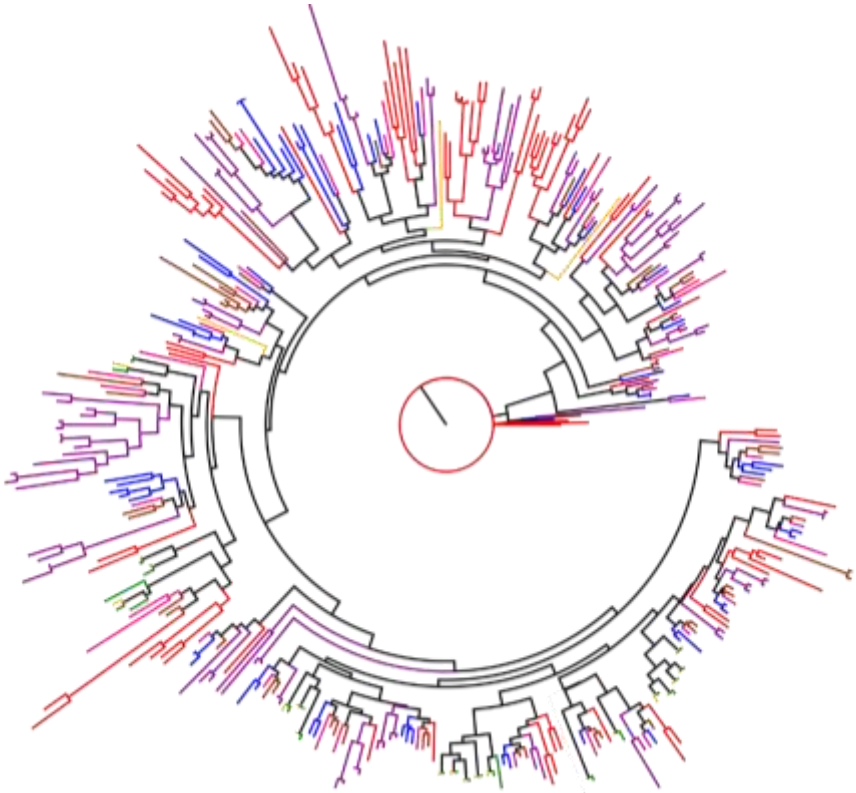

Figure 4: Phylogenetic Tree of PMEI. Violet: *Linum usitatissimum*; Brown: *Manihot esculenta*; Pink: *Ricinus communis*; Blue*: Populus trichocarpa*; Red*: Arabidopsis thaliana*; Green: *Musa acuminata* (A Genome); Yellow: *Musa balbisiana* (B Genome)

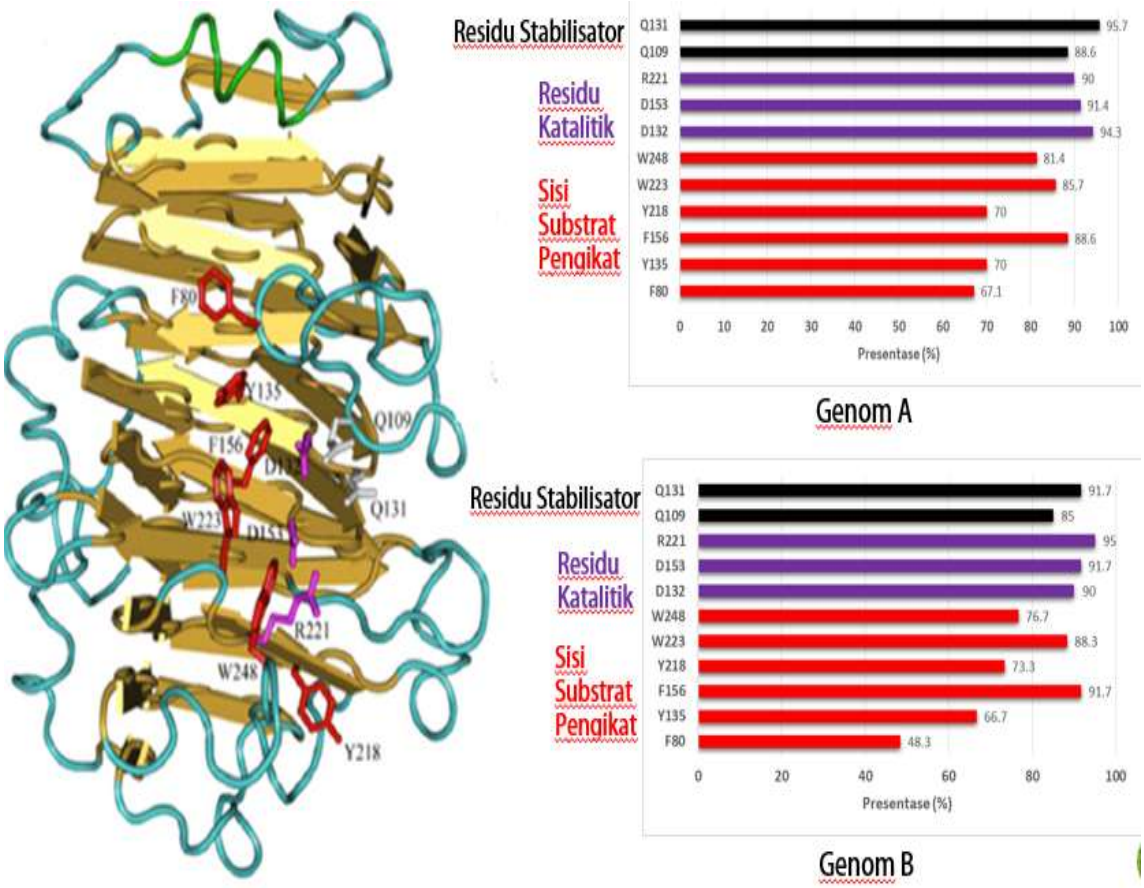

Figure 5: Conserved residues of PME in A and B Genome

The B group consists of PME type 1, which has a cleavage site and C consists of PME type 1, which has signal peptides but no cleavage side and two PME type 2 domains that did not have signal peptides and transmembrane domains. Furthermore, the D group is PME type 2, while the E group consists of PME type 1, which has a cleavage site and transmembrane domain and two PME type 2 domains that do not have signal peptides and transmembrane domains. The F group is the outgroup and all PME of other species except *Musa sp*. The relationship between genome A and B is orthologous[21,27].

In the PMEI phylogenetic tree (Figure 4), separate monocot and dicotyledonous species are clear, but no special characters were observed such as in the PME phylogenetic tree. Therefore, there are no monophyletic groups with special characters. Nevertheless, this PMEI tree remains valid because the bootstrap value of each branch is more than 70 %. Bootstrapping is a test of grouping stability in phylogenetic trees by repeating the sample trees through the number of replicates to be built. The overall relationship of the PMEI of A and B Genomes is orthologous[21].

Sustainable residues: The abundance of PME sustainable residues (Figure 5) in both banana genomes had similar results where the most abundant were catalytic residues and stabilizer residues with an average percentage of 90 %. Residues F80, Y135 and W223 were aromatic residues that interact with PMEI. Between the two genomes, the F80 was the least abundant followed by Y135, while W223 was the most conserved. The conserved residue in genome A for PMEI (Figure 6) is the group of cysteines that form disulfide bridges (100 %) and the second-highest preservation was for residues that were bound to PME.

Some residues did not have a residual abundance (0 % percentage), but the AA residues were replaced by other AA. In the aspartate position 109 (D109) in bananas, the AA threonine and serine class of polar AAs are not charged about 87 % of the time. The threonine position 73 (T73) is replaced by aspartate as much as 74 % of the time and the asparagine position 14 (N14) is replaced by the tyrosine AA as much as 83 % of the time.

The results of the sustainable residual abundance in PMEI B (Figure 7) are not much different from PMEI A; the highest preservation is the cysteine group that forms disulfide bridges and the second-highest sustainability was residues that interact with the PME residues.

Table 5
PME type 2 Characteristics from B Genome

S.N.	PME	Protein Characteristics			Subcellular Localisation				Conserved Residues		
		AA	Kda	pI	sp	tm	WolF PSORT	Plant-mPloc	AS	ST	CR
1	*MbPME1*	344	38.5	8.48	+	-	ch, ex, v	cw	3	2	3
2	*MbPME2*	379	41.5	9.13	+	-	ch, v, n, m	cw	6	2	3
3	*MbPME3*	297	33.3	9.49	-	+	ch, m, cy	cw	3	1	3
4	*MbPME5*	483	53.5	7.7	+	+	pl, g_pl, g, n, m, v	cw	4	2	3
5	*MbPME6*	326	36.3	9.35	+	+	v, ch, n, m, ex, er	cw	3	1	2
6	*MbPME12*	248	27	9.23	-	-	cy, ch	cw	4	2	2
7	*MbPME14*	673	73.4	6.63	-	-	er, pl, ex, v, ch, n, cy	cw	3	2	3
8	*MbPME17*	321	35.7	7.74	+	+	v, ex, ch, g	cw	4	2	3
9	*MbPME21*	352	38.3	8.79	+	+	ex, ch	cw	3	2	3
10	*MbPME25*	406	44.3	6.14	+	+	v, ex, ch, pl	cw	3	2	3
11	*MbPME26*	377	41.6	9.11	+	-	ch, v	cw	6	2	3
12	*MbPME27*	348	37.8	5.65	+	+	ch, ex, er, er_pl, m, pl	cw	2	1	3
13	*MbPME33*	1014	113	6.56	+	-	v, g, ch, n	cw	5	2	3
14	*MbPME35*	326	36	5.44	+	-	ch, ex	cw	3	1	3
15	*MbPME36*	285	32.1	6.66	+	-	ch, v, g, n, ex	cw	0	1	1
16	*MbPME37*	299	33.6	8.76	+	-	ch, v, cy	cw	3	1	3
17	*MbPME38*	344	38	7.12	-	-	n, ct, cy	cw	3	0	2
18	*MbPME43*	364	39.3	6.38	+	-	ex, v, n, cy, ch, m	cw	1	0	1
19	*MbPME44*	405	45	9.35	-	-	ch, cy, m	cw	6	2	3
20	*MbPME51*	247	27.1	8.59	+	+	pl, ex, v, g	cw	1	2	2
21	*MbPME55*	335	37.6	8.83	+	+	v, ch, ex,er, g	cw	3	2	3
22	*MbPME59*	423	48.3	5.37	-	-	ch, n, cy, pl	cw	4	2	3

SP: Signal Peptides; TM: Transmembrane Domain. Subcellular Localisation: ch: chloroplast; cw: cell wall; cy: cytosol; er: endoplasm reticulum; ex: extracellular/cell wall; g: golgi; m: mitochondria; n: nucleus; pl: plasma membrane; v: vacuola; pe: peroxisome; ct: cytoskeleton. (AS): Active Sites (max. 6); (ST): Stabilizer Residues (max. 2). CR: Catalytic Residues (max. 6).

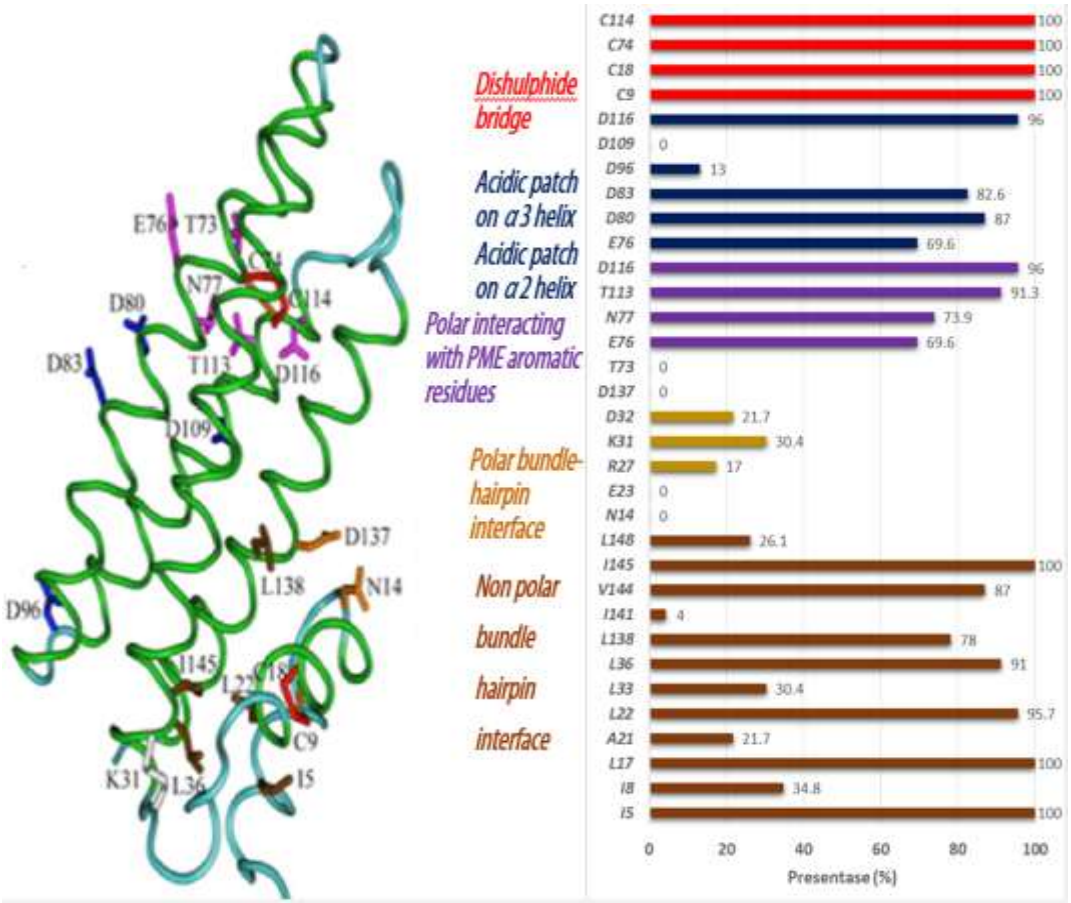

Figure 6: Conserved residues of PMEI in A Genome

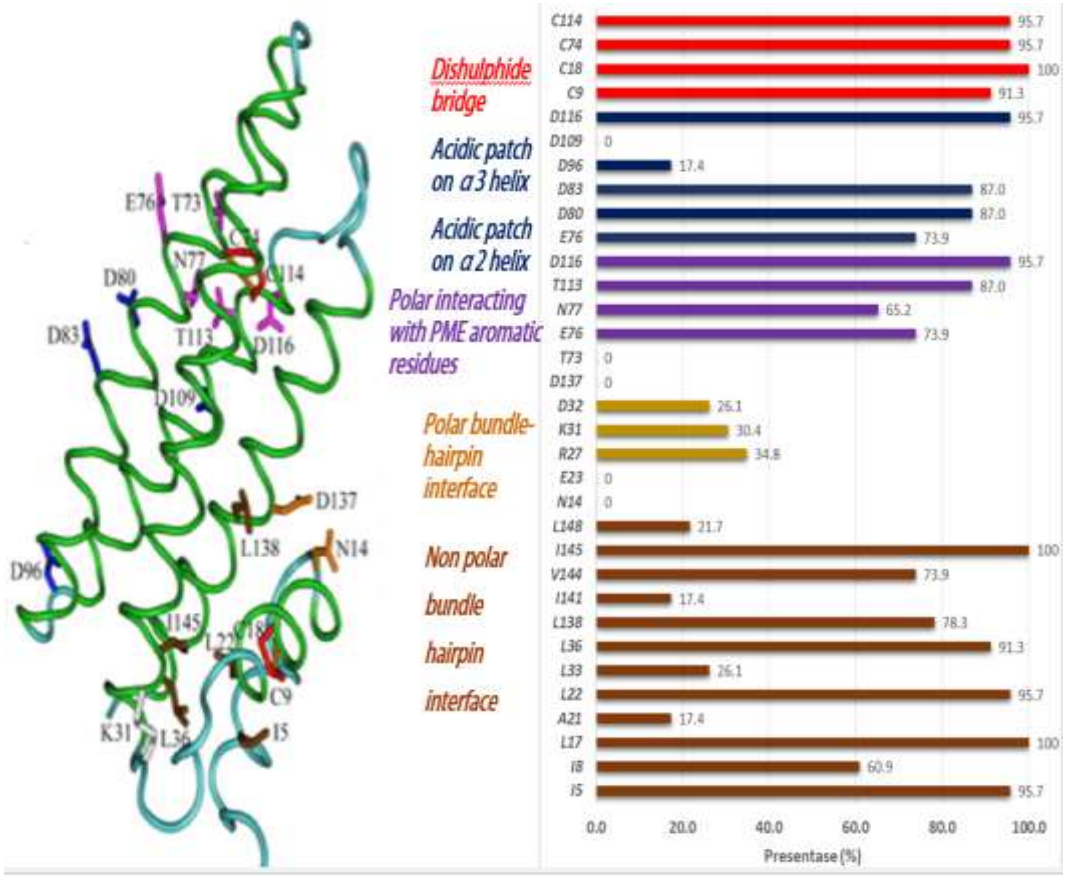

Figure 7: Conserved residues of PMEI in B Genome

Table 6
PMEI Characteristics from B Genome

S.N.	PMEI	Protein Characteristics			Subcellular Localisation				Conserved Residues					
		AA	Kda	pI	SP	TM	WolF PSORT	Plant-mPloc	*	§	□	£2	£3	¥
1	*MbPMEI1*	196	21.1	10.05	+	-	ch, v, ex	cm	7	1	4	3	1	4
2	*MbPMEI2*	204	21.1	8.33	+	+	ch	cm	8	0	3	2	1	4
3	*MbPMEI3*	214	23	8.81	+	+	ch, n, ex	cm	6	0	4	3	1	4
4	*MbPMEI4*	200	21.5	5.56	+	-	ex, er, er_pl, pl, ch, m	cm	6	1	4	3	1	4
5	*MbPMEI5*	194	21	9.06	+	-	ch, m	cm	7	0	4	3	1	4
6	*MbPMEI6*	185	19.5	5.18	+	-	ch, ex	cm	9	1	2	3	1	3
7	*MbPMEI7*	201	21	8.9	+	+	ch, m	cm	6	0	4	2	1	4
8	*MbPMEI8*	194	21.1	9.89	+	+	ch, v, ex	cm	7	0	4	3	1	4
9	*MbPMEI9*	209	22	6.45	+	-	ch, m	cm	7	0	4	3	1	4
10	*MbPMEI10*	197	21	4.85	+	+	ch, m	cm	10	2	3	3	2	4
11	*MbPMEI11*	206	22	7.73	+	-	ex, ch	cm	6	0	4	3	1	4
12	*MbPMEI12*	277	28.6	6.04	+	-	ch, m, ex	**cm**	9	0	4	3	2	4
13	*MbPMEI13*	194	21	10.21	+	+	ch, cy, m	cm	8	1	3	3	1	4
14	*MbPMEI14*	166	17.8	5.52	+	-	ex, cy, ch, m	cm	8	3	1	0	1	1
15	*MbPMEI15*	179	19	4.86	+	-	ex, ch, n	cm	10	1	3	2	2	4
16	*MbPMEI16*	183	19.2	8.52	+	-	ch, n, m, ex,	cm	6	0	0	1	0	4
17	*MbPMEI17*	198	21.2	8.21	+	+	ex, ch, cy	cm	6	0	4	3	1	4
18	*MbPMEI18*	185	18.9	7.87	+	-	ch, m	cm	9	0	3	1	1	4
19	*MbPMEI19*	177	18.5	4.08	+	+	ex, v	cm	8	1	4	3	1	4
20	*MbPMEI20*	201	21.4	7.04	+	-	ex, ch, m	cm	6	0	3	3	1	4
21	*MbPMEI21*	230	25.3	11.24	-	-	ch, n	cm, n	7	1	5	3	1	4
22	*MbPMEI22*	198	21	7.78	+	-	ex, v, cy	cm	7	1	3	2	1	4
23	*MbPMEI23*	187	19.4	5.86	+	-	ch, ex, cy	cm	11	1	2	2	2	4

SP: Signal Peptides; TM: Transmembrane Domain. Subcellular Localisation: ch: chloroplast; cw: cell wall; cy: cytosol; er: endoplasm reticulum; ex: extracellular/cell wall; g: golgi; m: mitochondria; n: nucleus; pl: plasma membrane; v: vacuola; pe: peroxisome; ct: cytoskeleton. Conserved residues as follows: (*) non polar bundle-hairpin interface (max. 12); (§) polar bundle-hairpin interface (max. 6); (□) polar residues interact with PME aromatic residues (max. 5); (£2) acidic patch on α2 helix (max. 3); (£3) acidic patch on α3 helix (max. 3); (¥) cysteine for disulfide bridge (max. 4)

A group of residues such as the 109th aspartate was replaced by an uncharged polar amino acid group of 87 %, then in threonine, the 73rd position was replaced by an aspartate of a negatively-charged protein group of 74 % and position 14 was replaced by an aromatic non-polar aromatic tyrosine group of 78 %. In the genome A and B PMEI, residues E23 and D137 also have 0 % abundance in bananas, but the replacement residue does not replace in full because the percentage is less than 50 %.

Conclusion

We found a great diversity of the PME and PMEI gene families in banana A and B genomes. Additionally, we characterized the subcellular localization of the proteins, conserved residues and phylogenetic relationship between A and B genomes. This information will allow relevant genes to be identified for future banana improvements.

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