APPENDICES

APPENDIX A

Preparation of the chemical reagents

A1. Preparation of the chemical reagents for determination of GABA and amino acids

1. **0.3% Hydroxynaphaldehyde (HN), 100 ml**

   0.3 g of HN was dissolved with methanol and make up to 100 ml.

2. **Borax buffer pH8, 100 ml**

   a. 0.75 g boric acid

   b. 4.75 g Sodium tetraborate decahydrate (borax)

   c. Deionized water 250 ml

3. **1000 ppm Standard GABA**

   0.1 g of GABA was dissolved in water and made up to 100 ml.

4. **0.1% Formic acid (Mobile phase)**

   Pipet 1 ml of formic acid into 1000 ml of the milli-Q water

A2. Preparation of the chemical reagents for Protein extraction

1. **Stock solution of 500 mM HEPES buffer pH 7.4 , 50 ml**

   a. 5. 96 g HEPES

   b. Add distillated to make 50 ml

2. **Stock solution of 0.5M Magnesium Choride , 50 ml**

   a. 5. 0825 g Magnesium Choride

   b. Add distillated to make 50 ml

3. **Stock solution of 500 mM EDTA-Na , 50 ml**

   a. 9.306 g EDTA-Na

   b. Add distillated to make 50 ml

4. **Stock solution of 10 mM Leupeptin , 1 ml**

   a. 5 mg Leupeptin

   b. Add distillated to make 1 ml

   Stored at -30 ^{o}C

5. Pre-extraction buffer, 100 ml

a. 500 mM HEPES buffer pH 7.4	10	ml
b. 0.5M Magnesium Choride	2	ml
c. 500 mM EDTA-Na	0.2	ml
d. 10% glycerol	10	ml
e. Insoluble PVP	5	g

d. Add distillated water to a total volume of 100 ml

PVP is insoluble reagent, Should be lastly added.

Keep at Room temperature.

6. Extraction buffer, 10 ml

a. Pre-extraction buffer	10	ml
b. 5mM DTT	50	µl
c. 10 mM Leupeptin	10	µl

Keep at 4 ^{o}C in the refrigerator

A 3. Preparation of the chemical reagents for SDS-Polycrylamide gel

1. 30% (w/v) Acylamide, 0.8 %(w/v) bis-acylramide , 100 ml

a. 30 g acylamide

b. 0.8 g bis-acylramide

Add distillated to make 100 ml and stir until thoroughly mixed.

Work under hood and keep acrylamide solution covered with parafilm until completely dissolved.

c. Can be stored for months in the refrigerator

2. 4X Tris/SDS pH 8.8, 500 ml

a. Weight out Tris-hydroxy methyl aminomethane (This base) 91 g

b. Add to 300 ml distillated water

c. Add concentrated HCl slowly to pH 8.8

d. Add distillated water to a total volume of 500 ml

3. 4X Tris/SDS pH 6.8, 200 ml

a. Weight out This base 12.1 g

b. Add to 100 ml distillated water

c. Add concentrated HCl slowly to pH 6.8

d. Add distilled water to a total volume of 200 ml

Stable for months in the refrigerator.

4. 2X SDS loading buffer 10 ml

a. 4X Tris/SDS pH 6.8 2.5 ml

b. 100% glycerol 2 ml

c. 0.4 g SDS

d. 0.31 g DTT

e. 0.1 mg bromophenol blue

Add distilled water 5 ml, shake until thoroughly mixed and made up the volume to 10 ml Stored at -70 ^{o}C

5. 10% Ammonium persulphate, 1 ml

a. 0.1 g ammonium persulphate

b. Add distilled water 1 ml , shake until thoroughly mixed.

Fresh preparation before used.

6. 4X Tris running buffer 1 L

a. 12 g Tris base

b. 57.6 g glycine

c. Add distilled water 500 ml, shake until thoroughly mixed and made up the volume to 1000 ml

7. 1X SDS running buffer 400 ml

a. 4X Tris running buffer 100 ml

b. 20% SDS (20 g SDS was dissolved in 100 ml distilled water) 2 ml

c. Add distilled water to make 400 ml

8. 1X SDS running buffer 400 ml

a. 4X Tris running buffer	100	ml
b. 20% SDS	2	ml
c. Add distilled water to make	400	ml

9. Staining solution, 1000 ml

a. Methanol	400	ml
b. Acetic acid	100	ml
c. 1 g Coomassie Blue R-250		
d. Add distilled water to make	1000	ml

9. Destaining solution, 1000 ml

a. Methanol	300	ml
b. Acetic acid	100	ml
c. Add distillated water to make	1000	ml

A 4. Preparation of the chemical reagents for Western blotting

1. Blotting solution, 1000 ml

a. 12.1 g Tris-base

b. 14.4 g glycine

c. 10% SDS 5 ml

d. Methanol 200 ml

e. Add distillated water to make 1000 ml, pH should be between 8.1-8.4 without adjustment.

2. 10X TBS pH 7.5, 1000 ml

a. 60.5 g Tris-base

b. 87.7 g Sodium chloride

c. Add distillated water to make 1000 ml

3. 1X TBS pH 7.5, 1000 ml

a. 10X TBS pH 7.5,	100	ml
b. Add distillated water to make	1000	ml

4. TBS-T, 1000 ml

a. 1X TBS	1000	ml
b. Tween 20	1	ml

A 5. Preparation of the chemical reagents for Proteome analysis

1. Rehydration buffer, 395 µl

a. 0.1712 g Urea

b. 0.062 g Thiourea

c. TritonX 100 (20%)	132.34	µl
d. DTT 1 M	8.14	µl

e. Add distillated water to make 395 µl

2. Polyacrylamide gel, 18 × 35 cm (for 2 gel)

a. 30% acrylamide	54.167	ml
b. 1.5 M Tris-Cl pH 8.8	32.5	ml
c. 10% SDS	1.3	ml
d. TEMED	43.3	µl
e. 10% Ammonium persulphate	650	µl
d. Distillated water	43.3	ml

3. DTT equilibrated solution (for 2 gel)

a. 0.5M Tris-Cl pH 6.8	4	ml
b. 10% SDS	8	ml
c. 100% glycerol	12	ml

d. 14.40 g Urea

e. 0.4 g DTT (add before used about 20 min)

f. Stirred until thoroughly mixed and add distillated water to make 40 ml

4. Iodoacetamide equilibrated solution (for 2 gel)

a. 1.5M Tris-Cl pH 6.8	1332	µl
b. 10% SDS	8	ml
c. 100% glycerol	12	ml

d. 14.41 g Urea

e. 0.4 g Iodoacetamide (add before used about 20 min)

f. Stirred until thoroughly mixed and add distillated water to make 40 ml

5. SDS Running buffer 18 × 35 cm (for 2 gel), 4 L

a. 12.114 g Tris-base

b. 57. 6536 g glycine

c. 4.0379 g SDS

d. Add distillated water to make 40 ml

e. Stirred until completely dissolved

6. 0.5% Agarose, 30 ml

a. 0.15 g agarose

b. Bromophenol blue	0.5	ml
d. Add distillated water to make	30	ml

e. Microwave for 2 min before used

7. Staining Solution 18 × 35 cm (for 2 gel), 1 L

a. Methanol	200	ml
b. Acetic acid	35	ml
c. 0.5 g Coomassie blue R 250		
d. Add distillated water to make	1000	ml

8. Destaining Solution I 18 × 35 cm (for 2 gel), 700 ml

a. Methanol	280	ml
b. Acetic acid	49	ml
c. Add distillated water to make	700	ml

9. Destaining Solution II 18 × 35 cm (for 2 gel), 1000 ml

a. Methanol	50	ml
b. Acetic acid	70	ml
c. Add distillated water to make	1000	ml

10. 1M NH_4HCO_3, 1 ml

a. 0.0791 g NH_4HCO_3

b. Add distillated water to make 1 ml

11. 25mM NH_4HCO_3 100 µl

a. 1M NH_4HCO_3	2.5	µl

b. Add distillated water to make 100 µl

12. 50%ACN 25 mM NH_4HCO_3, 100 µl

a. 100% acetonitrile (ACN)	50	µl
b. 1M NH_4HCO_3	2.5	µl
c. Add distillated water to make	100	µl

13. **Trypsin buffer,** 200 µl

a. 20 ug Trypsin

b. Add distillated water to make 200 µl

14. 10% Trypsin-0.1M NH_4HCO_3 100 µl

a. Trypsin buffer	12.5	µl
b. 0.1M NH_4HCO_3	25	µl

c. Add distillated water to make 100 µl

15. 50%ACN-0.5%TFA, 1 ml

a. 100% ACN (Acetronitrile)	500	µl
b. 100% TFA (Trifluoroacetic acid)	5	µl

c. Add distillated water to make 1000 µl

16. Matrix 0.05% TFA, 1 ml

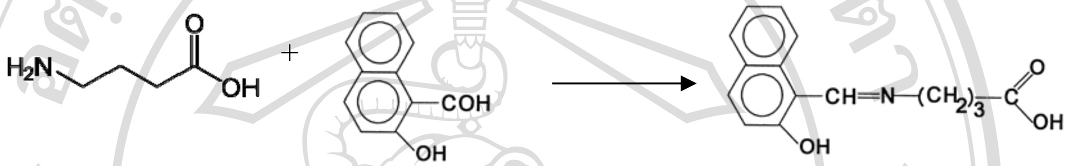
a. 0.007 g cyano -4-hydroxy cinnamic acid (CHCA)

b. 100% Acetronitrile	500	µl
c. 100% Trifluoroacetic acid (TFA)	5	µl
d. D.W. (filled up to)	1000	µl

APPENDIX B

Derivatization for the determination of GABA and amino acids

Crude extracted of rice containing GABA and amino acids was reacted with HN solution prior to LC-MS chromatography analysis. The description of derivatization method shown in **section 2.2.4, page 55.** The derivatization reaction shown in **Figure B.1** and the molecular weight of GABA and amino acids were shown in **Table B.1**



GABA (MW 103) HN (MW 172) GABA-HN (MW 257)

Figure B.1 GABA derivatization reaction

Table B.1 Molecular weight of GABA and 12 amino acids containing in rice sample before and after derivatization with HN

Type	Molecular weight		
	Before derivertization	**After derivertization**	**MS analysis**
GABA	103.12	257	258
Arginine	174.20	328	329
Asparagine	132.12	286	287
Serine	105.9	259	260
Glycine	75.07	229	230
Threonine	119.2	273	274
Glutamic acid	147.13	301	302
Tyrosine	181.19	335	336
Alanine	89.10	243	244
Tryptophan	204.13	358	359
Isoleucine	131.18	285	286
Leucine	131.18	285	286

APPENDIX C

Calculations

C 1. Measuring the content of GABA content

Ground sample 0.25 g in 4 ml 70% ethanol extract was used. Determination of the content of GABA was carried out by comparing the peak area with the peak area of the standard reference compounds obtained by LC-MS at the same condition. The calculation was done as followed.

C.1.1 Example peak area of injected example

Table C.1 Example of peak area of injected sample of non-germinated SPT1 cultivar (control)

SPT	Peak Area								
	Cultivation repeat 1 (1-30 January 2008)			Cultivation repeat 2 (1-30 Febuary 2008)			Cultivation repeat 3 (1-30 March 2008)		
	Inject1th	Inject 2nd	Average	Inject1th	Inject 2nd	Average	Inject1th	Inject 2nd	Average
	64.9039	109.081	64.9039	67.7820	60.29511	64.0385	65.4204	66.45436	65.9373

C.1.2 Calculation average area of sample compared to the standard curve

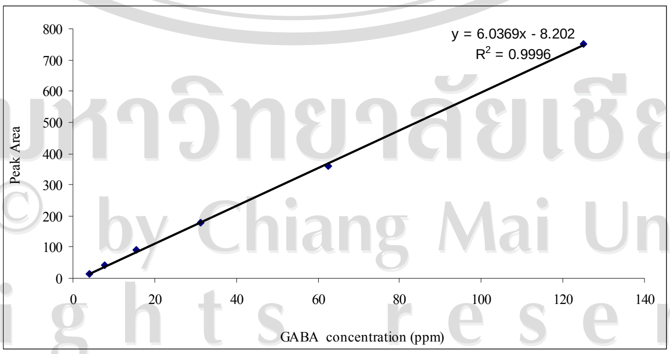


Figure C.1 Standard calibration curve of GABA standard

Example; Non- germinated SPT1 Cultivation repeat 1 (1-30 January 2008) presented average peak area ; **64.9039** (y)

While standard curve showed equation; y = 6.0369x - 8.202

Therefore; $x = \frac{y+8.202}{6.036}$

Average peak area ; 64.9039 (y)

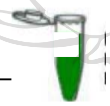
$x = \frac{\mathbf{64.9039} + 8.202}{6.036} = 12.1098428$ ppm

Therefore in this step; non- germinated SPT1 contain GABA = 12.11 ppm

C.1.3 Back calculation

Sample preparation showed in the Figure **C.1.3**.

Pipett 1 ml



10 µl → inject

0.25 g in total 4 ml 70% ethanol → 12.11 × 2 = 24.22 ppm

derivatizing agent 1 ml total 2 ml → 12.11 ppm

= 12.11 ppm (obtained from step **B.1.2**) → 12.11 ppm

Therefore; the sample contained GABA 24.22 ppm in total volume 4 ml

	Solution 1000	ml	has GABA	24.22 mg
If;	Solution 4	ml	has GABA	$\frac{24.22 \text{ mg} \times 4 \text{ ml}}{1000 \text{ ml}} = 0.0969$ mg

Weight rice sample 0.25 g for extraction in total volume 4 ml

Therefore;	rice sample 0.25	g	has GABA	0.0969 mg
If;	rice sample 1	g	has GABA	$\frac{0.0969 \times 1 \text{ g}}{0.25 \text{ g}}$ = **0.387 mg/g**

In summay;

SPT Cultivation repeat **1** (1-30 January 2008) contain GABA = **0.387** mg/g

SPT Cultivation repeat **2** (1-30 Febuary 2008) contain GABA = **0.383** mg/g

SPT Cultivation repeat **3** (1-30 March 2008) contain GABA = **0.392** mg/g

Average = **0.387 + 0.383 + 0.392** mg/g

= **0.39** mg/g ± **SD 0.01**

The data was plot in graph in **Figure 3.4** of Chapter 3 represented SPT1 (control) data. For the amino acids concentrations the data calculation same as the calculated GABA contents as describe above.

C 2. Measuring the GAD activity

The GAD activity was calculated according to their reaction and subsequently injected into LC-MS. Determination of the content of GABA as a product of reaction was carried out by comparing the peak area with the peak area of the standard curve The peak area of GABA in reaction was subtracted from the peak area of crude enzyme which contains GABA as endogenous product (control).

The calculation was done as followed.

C.2.1 Example peak area of injected example

Chromatogram and peak area of GABA produced from crude GAD enzyme

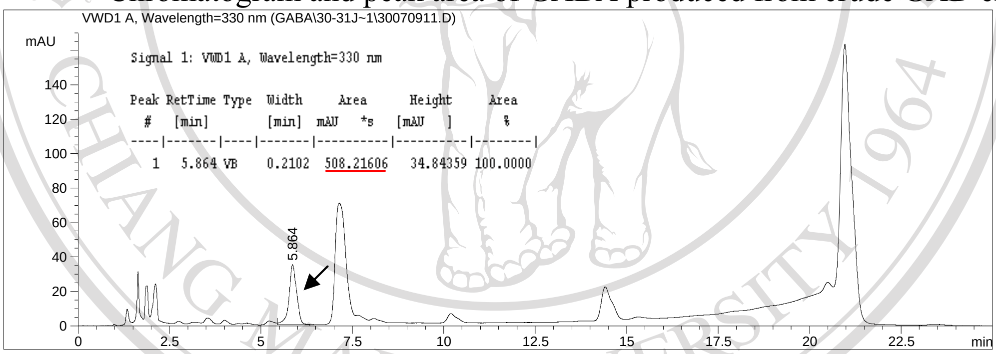


Figure C.2 Chromatogram and peak area of GABA produced from crude GAD enzyme within 10 min of KDML cultivars at 5 germination day.

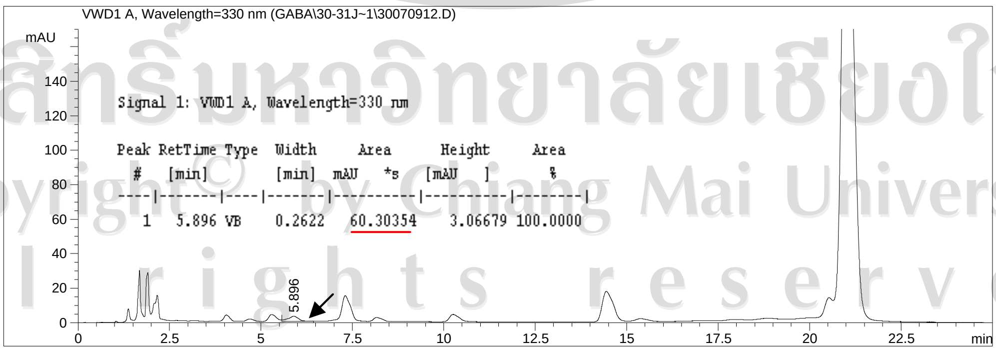


Figure C.3 Chromatogram and peak area of crude GAD enzyme containing endogenous GABA .

Area of GABA from enzyme reaction = 508.21606 (Area 1) - 60.30354 (Area2)

= **447.91252**

C.2.2 GABA calculation from standard curve

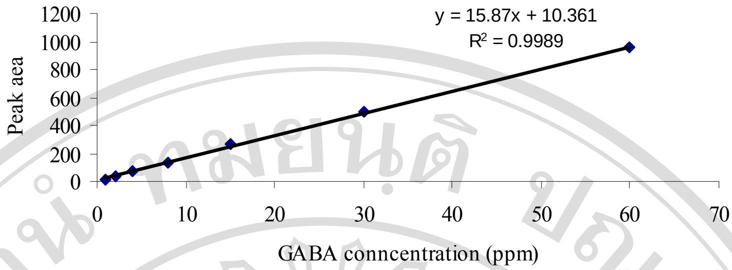


Figure C.4 Standard curve of GABA standard for GAD activity assay

Example;

Area of GABA from enzyme reaction = 508.21606 - 60.30354

= **447.91252** (obtained from step **C.1.1**)

While, standard curve showed equation; y = 15.87x + 10.361

Therefore;

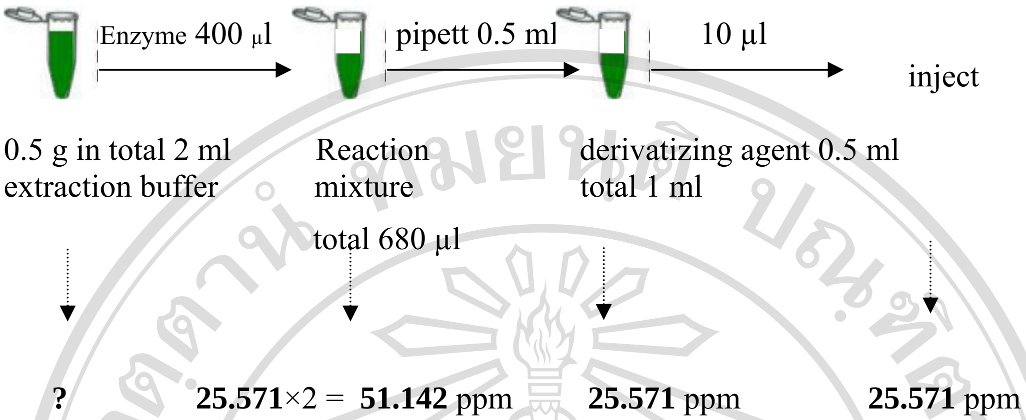
$$x = \frac{y - 10.361}{15.87}$$

Average peak area ; **447.91252** (y)

$$x = \frac{\mathbf{447.91252} - 10.361}{15.87} = 25.571$$

Therefore in this step; Non- germinated SPT1 contain GABA = **25.571** ppm

C.2.3 Back calculation



Therefore; the sample contained GABA 51.142 ppm in total volume 680 μl

Solution 1000	ml	has GABA	=	**51.142** mg
Total Crude enzyme 2	ml	can produce GABA	=	**0.092** mg

Therefore; **0.092** mg $= \dfrac{0.092\times10^{-3}}{10^{-6}}$ ug $= \dfrac{92.05\mu g}{103 \text{ (MW of GABA)}} = 0.89$ μmol

In this study, the reaction was carried out 10 min

Therefore, Crude enzyme 2 ml can produce GABA = 0.89 μmol /10 min

One unit of GAD activity was defined as release of 1 μmol of GABA produced per 30 min at 40°C.

Therefore; Crude enzyme 2 ml can produce GABA $= \dfrac{0.89 \ \mu mol \times 30 \ min}{10 \ min}$

= 2.67* **Unit**

(* Example value shown in **Figure 3.12a** of leave for SPT1 cultivars at 5 germination day)

C.3 Protein concentrations

Protein concentrations in rice extract samples were measured and prepared as shown in **Table C.3.1**. Determination of protein was carried out by comparing the OD_{595} value with OD_{595} of the standard curve (**Figure C.5**)

Table C.2 The amount of solution for the Bradford assay for calculation

Solution	**Volume (µl)**							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
BSA 100 ug/ul	0	20	40	60	80	-	-	-
Water	800	780	760	740	720	798	798	798
Crude extracted	-	-	-	-	-	2	2	2
Coomassie BioRad	200	200	200	200	200	200	200	200
OD_{595}	0.582	0.714	0.811	0.910	0.1035	1.019	1.013	0.112

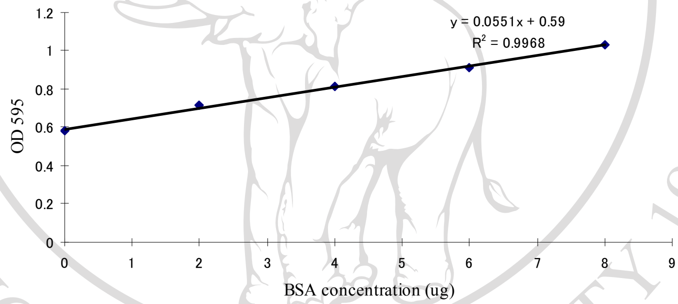


Figure C.5 Standard curve of BSA standard

Example;

Average OD $_{595}$ of sample $= \frac{1.019 + 1.013 + 1.112}{3} = 1.048$

Standard curve of BSA showed equation; $y = 0.0551x + 0.59$

Therefore; $x = \frac{y - 0.59}{0.0551}$

Average peak area ; 1.048 (y) $x = \frac{1.048 - 0.59}{0.0551}$

$x = 8.312$ ug/2 µl

Protein concentration in sample = 4.15 µg/µl

Calculate for proteomic analysis ; 470 µg = pipett 113. 25 µl
Calculate for subjected into SDS-PAGE well; 10 µg = pipett 2.40 µl

APPENDIX D

Chromatogram of Liquid chromatography -Mass Spectrum

D1. Chromatogram of standard GABA

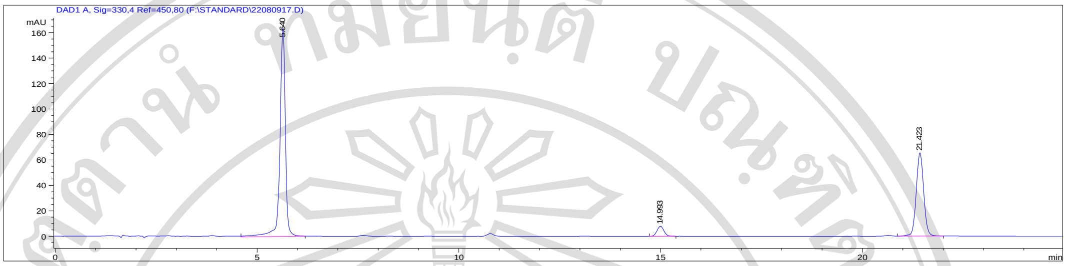


Figure D1.1.1 Standard GABA 250 ppm

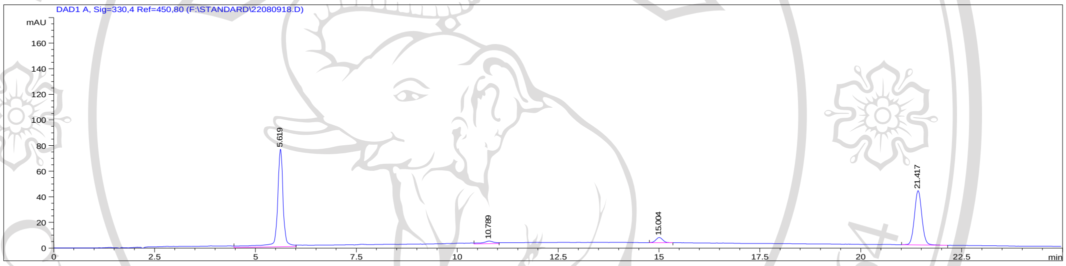


Figure D1.1.2 Standard GABA 125 ppm

Figure D1.1.3 Standard GABA 50 ppm

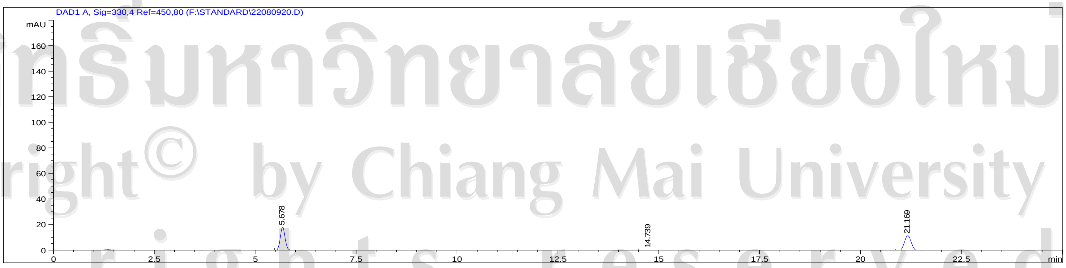


Figure D1.1.4 Standard GABA 10 ppm

D 2. Chromatogram and mass spectrum of samples for GABA

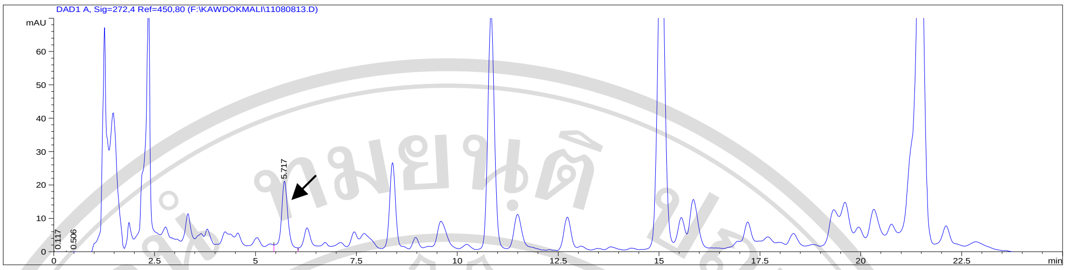


Figure D2.1.1 Chromatogram of germinated rice grains containing GABA

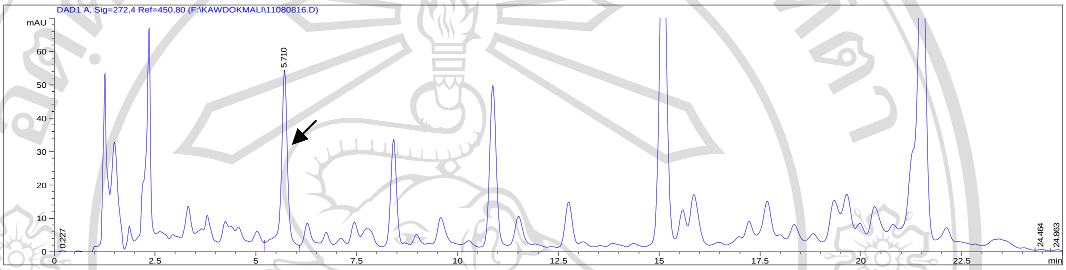


Figure D 2.1.2 Chromatogram of young leaves containing GABA

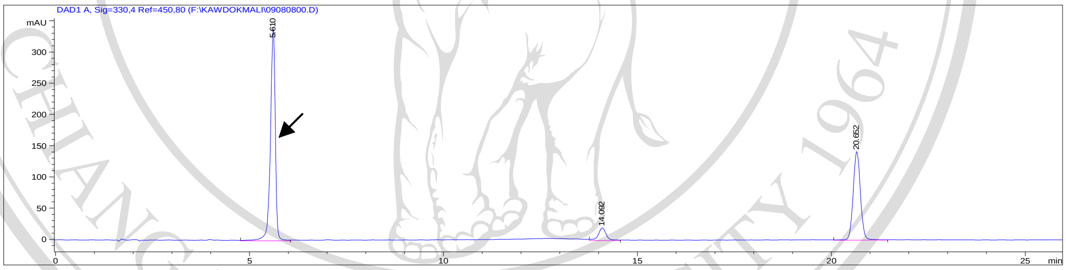


Figure D 2.1.3 Chromatogram of GABA standard

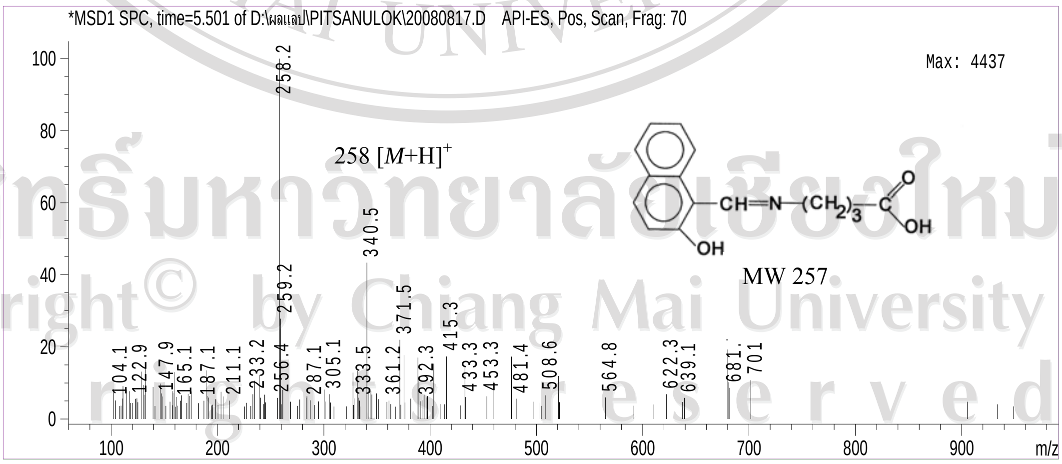


Figure D 2.1.4 Mass spectrum of samples containing GABA

D 3. Chromatogram and mass spectrum of samples for amino acids

3.1 Arginine

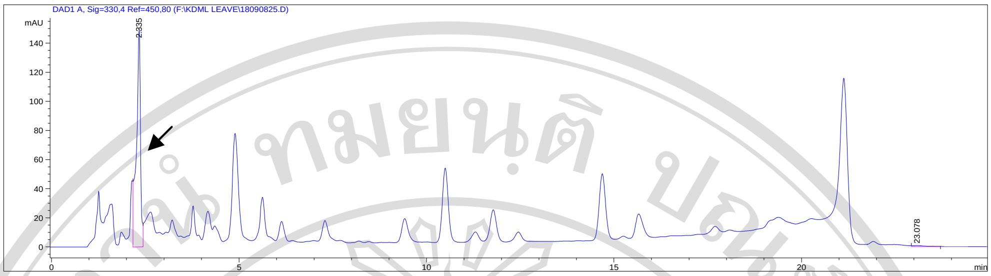


Figure D 3.1.1 Chromatograms of young leaves containing arginine

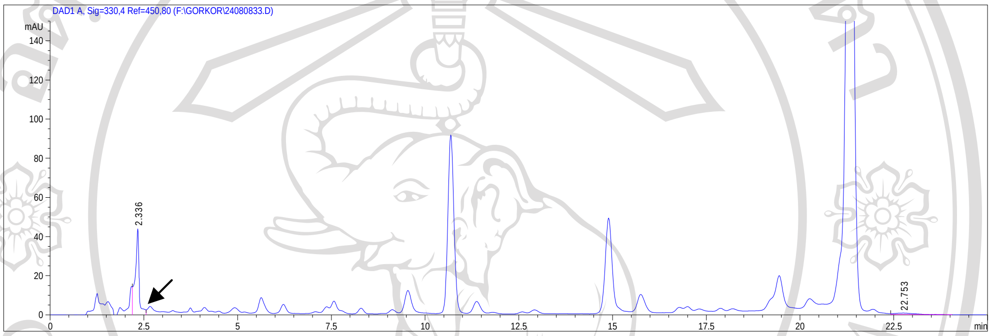


Figure D 3.1.2 Chromatograms of germinated rice grains containing arginine

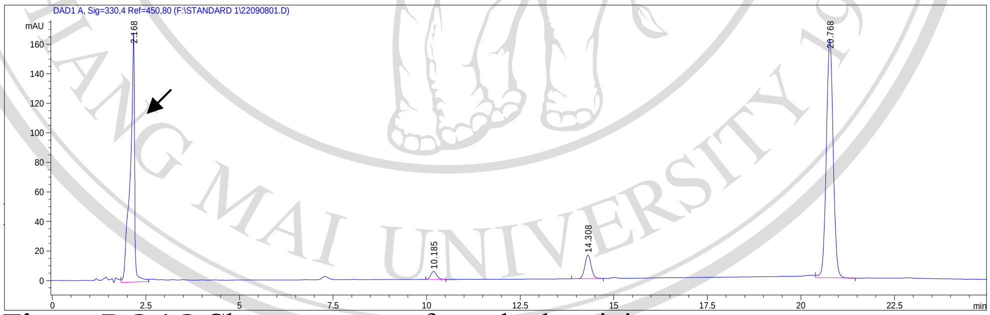


Figure D 3.1.3 Chromatogram of standard arginine

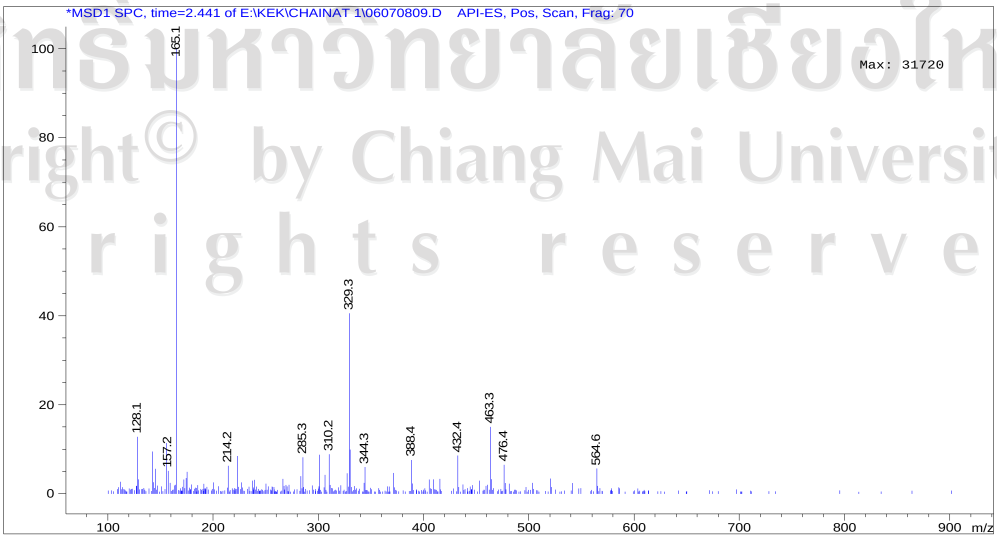


Figure D 3.1.4 Masspectrum sample containing arginine

3.2 Asparagine

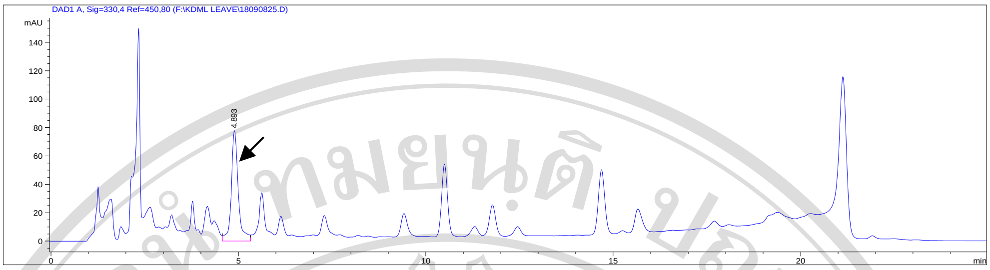


Figure D 3.2.1 Chromatograms of young leaves containing asparagine

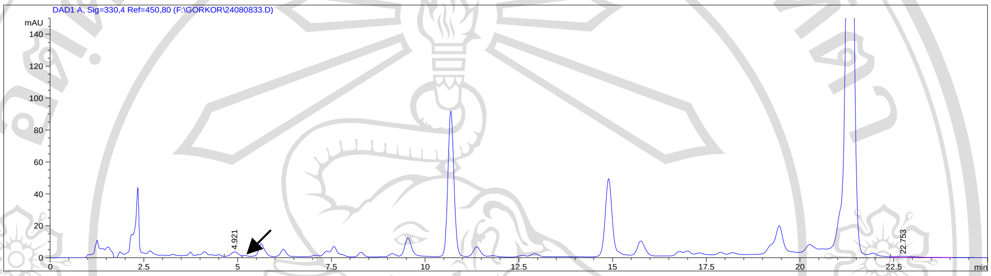


Figure D 3.2.2 Chromatograms of germinated rice grains containing asparagine

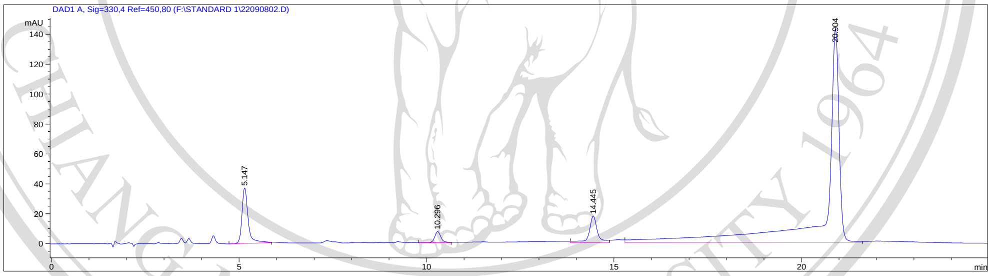


Figure D 3.2.3 Chromatograms of standard of asparagine

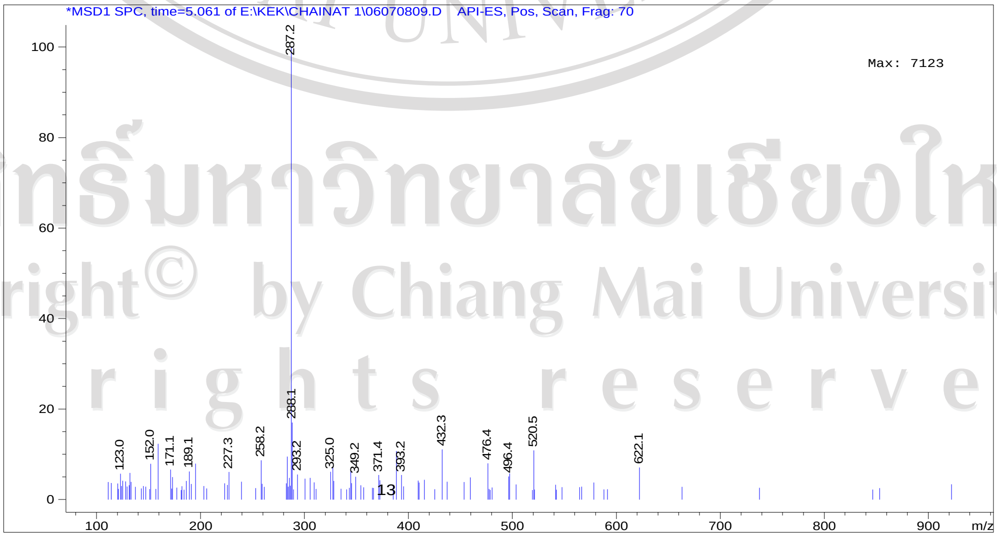


Figure D 3.2.4 Mass spectrum sample containing asparagine

3.3 Serine

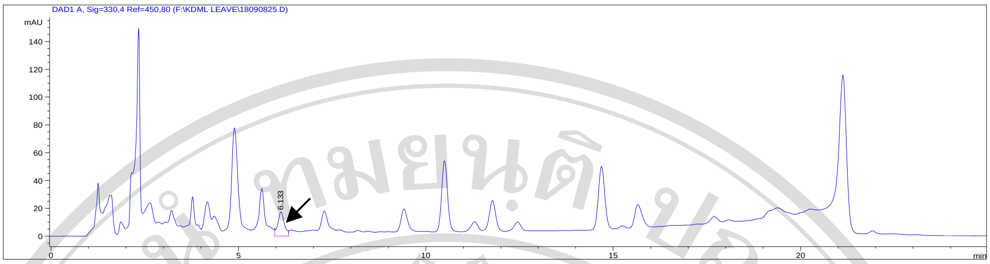


Figure D 3.3.1 Chromatograms of young leaves containing serine

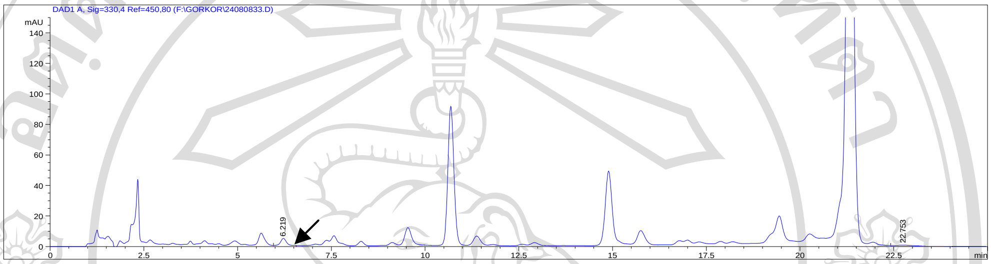


Figure D 3.3.2 Chromatograms of germinated rice grains containing serine

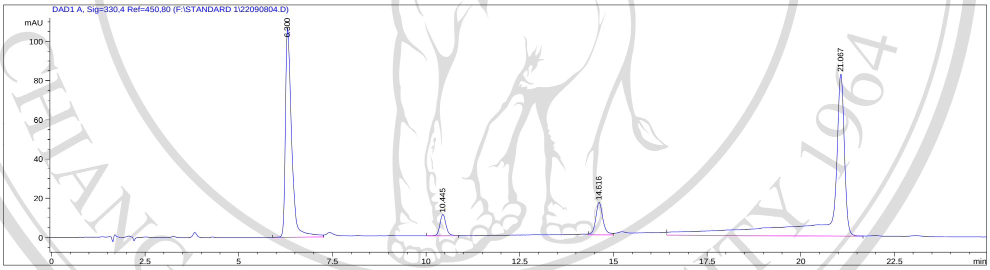


Figure D 3.3.3 Chromatograms of standard of serine

Figure D 3.3.4 Mass spectrum sample containing serine

3.4 Glycine

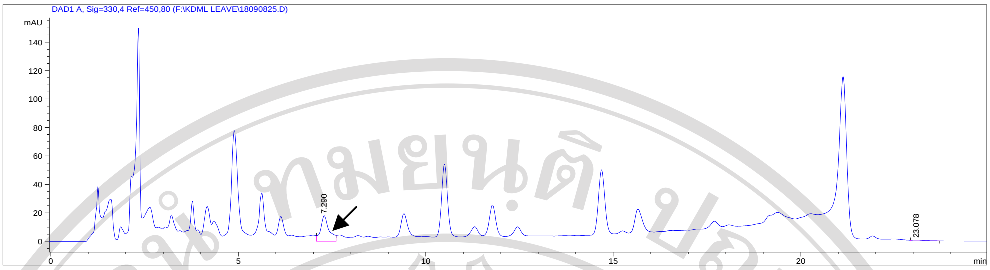


Figure D 3.4.1 Chromatograms of young leaves containing glycine

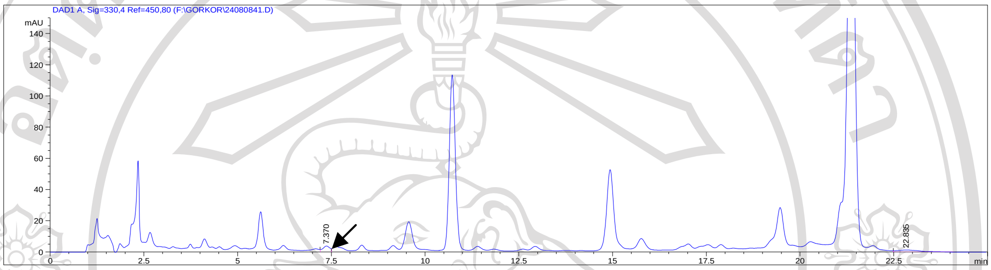


Figure D 3.4.2 Chromatograms of germinated rice grains containing glycine

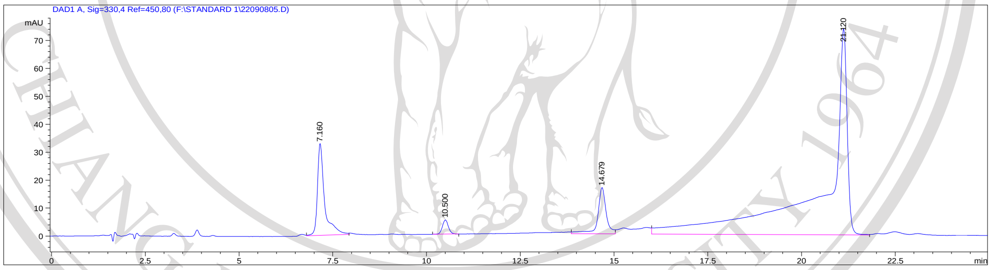


Figure D 3.4.3 Chromatograms of standard of glycine

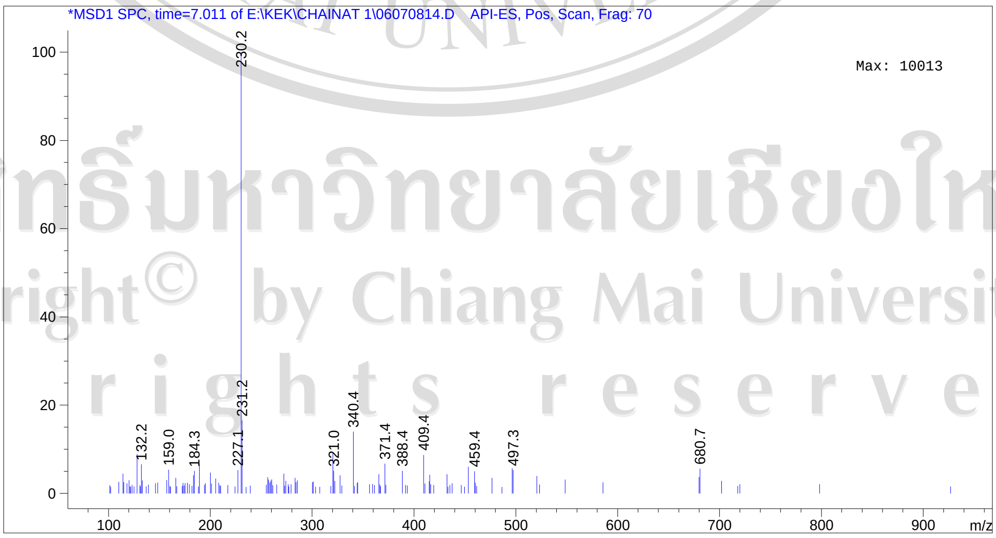


Figure D 3.4.4 Mass spectrum sample containing glycine

3.5 Glutamic acid

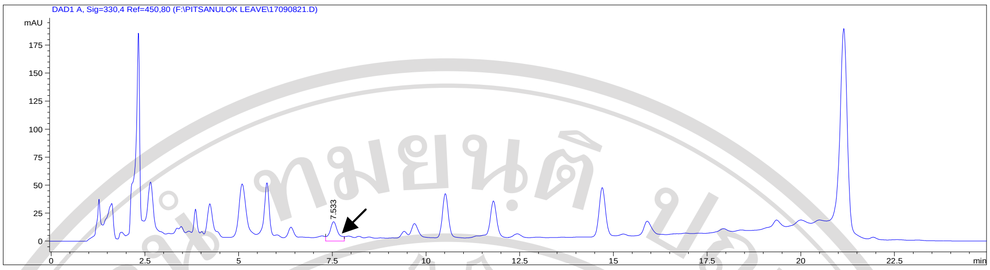


Figure D 3.5.1 Chromatograms of young leaves containing glutamic acid

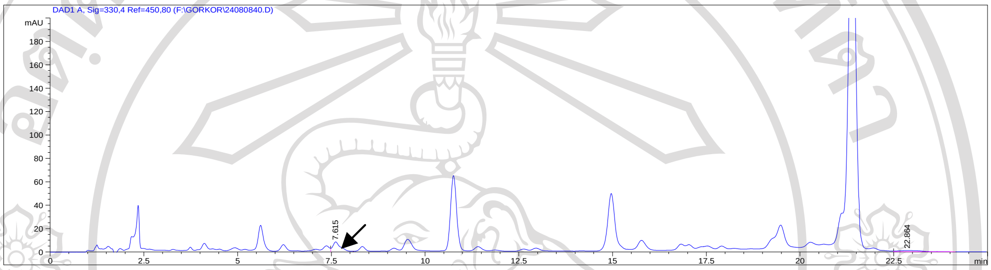


Figure D 3.5.2 Chromatograms of germinated rice grains containing glutamic acid

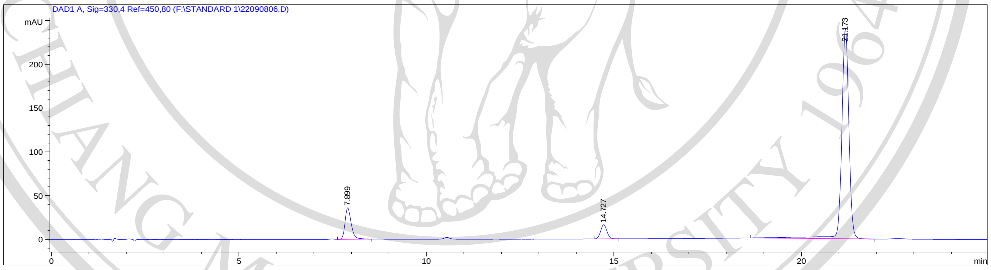


Figure D 3.5.3 Chromatograms of standard of glutamic acid

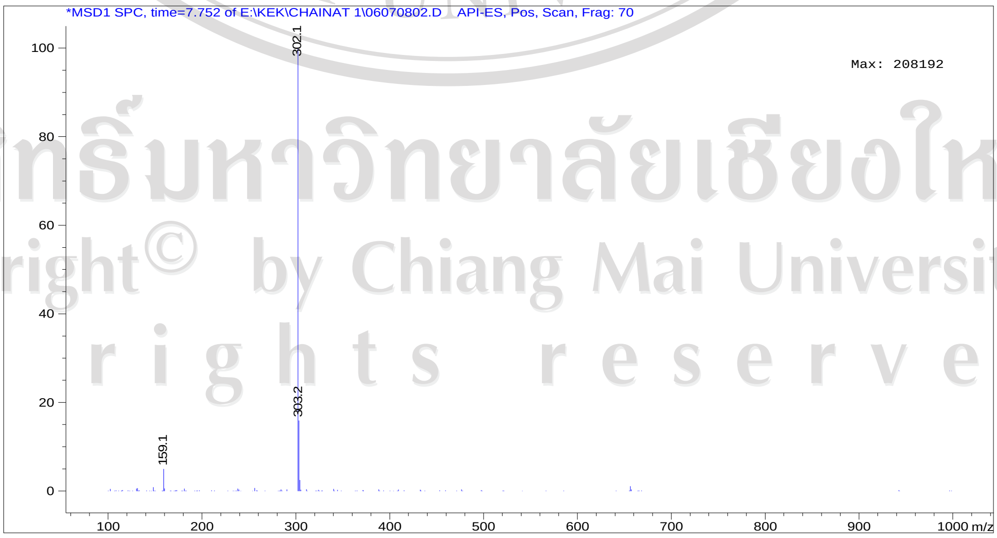


Figure D 3.5.4 Mass spectrum of sample containing glutamic acid

3.6 Tyrosine

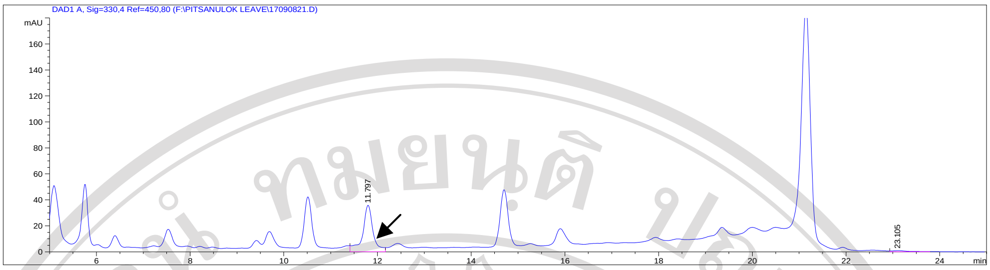


Figure D 3.6.1 Chromatograms of young leaves containing tyrosine

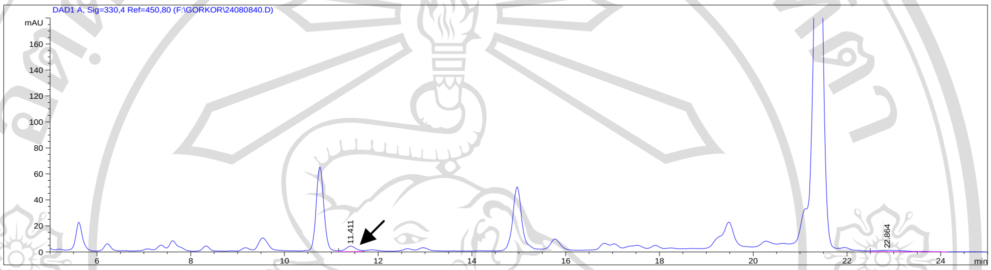


Figure D 3.6.2 Chromatograms of germinated rice grains containing tyrosine

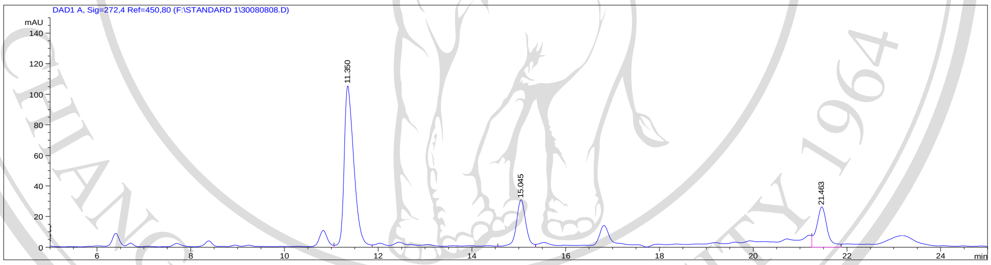


Figure D 3.6.3 Chromatograms of standard of tyrosine

Figure D 3.6.4 Mass spectrum of sample containing tyrosine

3.7 Alanine

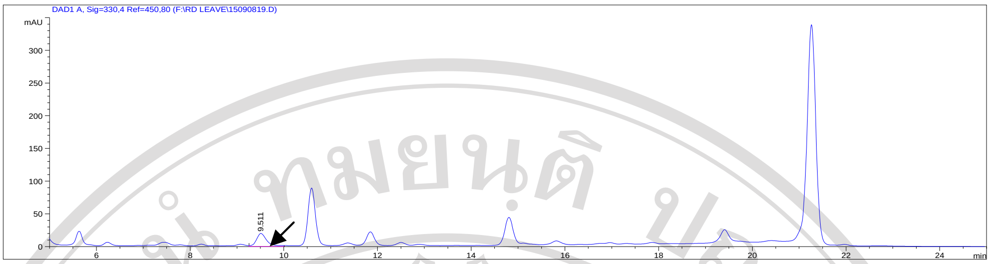


Figure D 3.7.1 Chromatograms of young leaves containing alanine

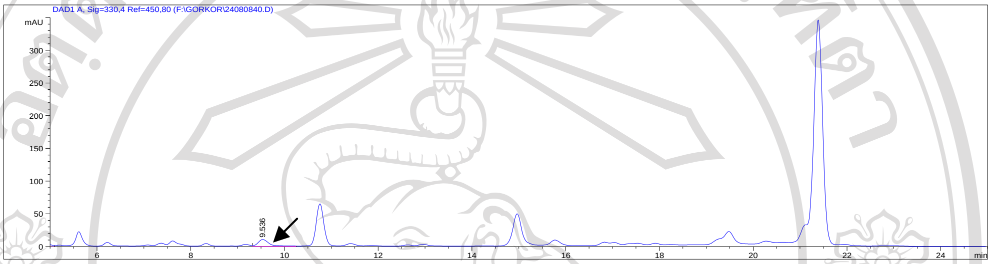


Figure D 3.7.2 Chromatograms of germinated rice grains containing alanine

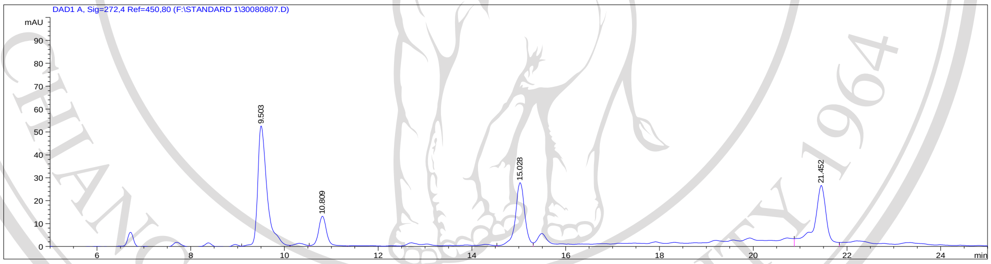


Figure D 3.7.3 Chromatograms of standard of alanine

Figure D 3.7.3 Mass spectrum of sample containing alanine

3.8 Valine

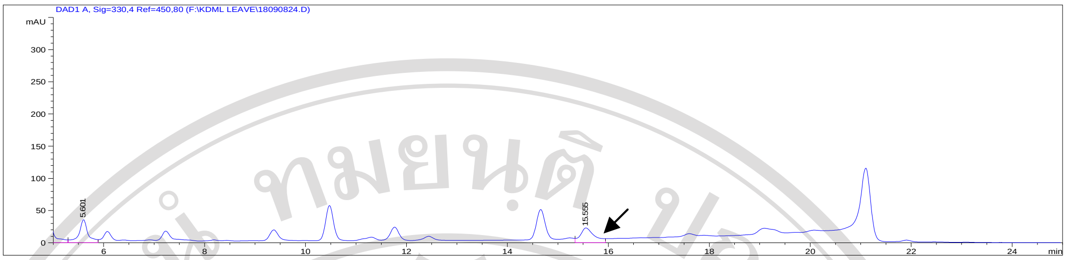


Figure D 3.8.1 Chromatograms of young leaves containing valine

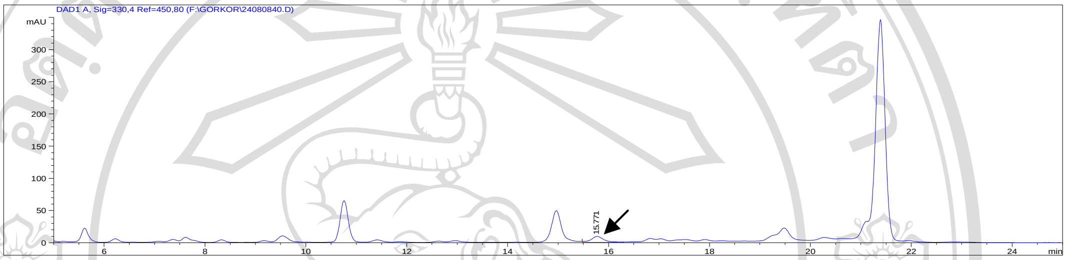


Figure D 3.8.2 Chromatograms of germinated rice grains containing valine

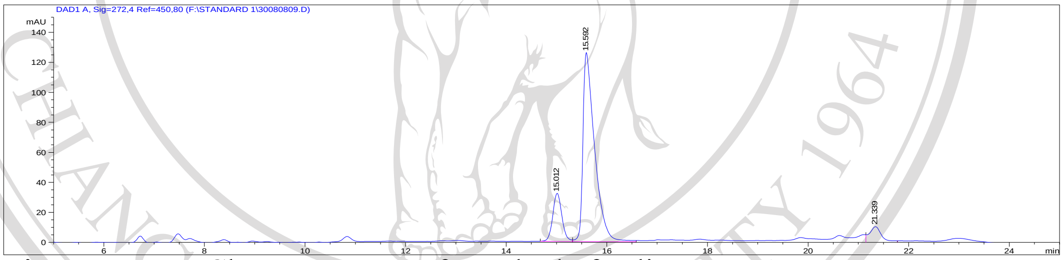


Figure D 3.8.3 Chromatograms of standard of valine

Figure D 3.8.4 Mass spectrum of sample containing valine

3.9 Tryptophan

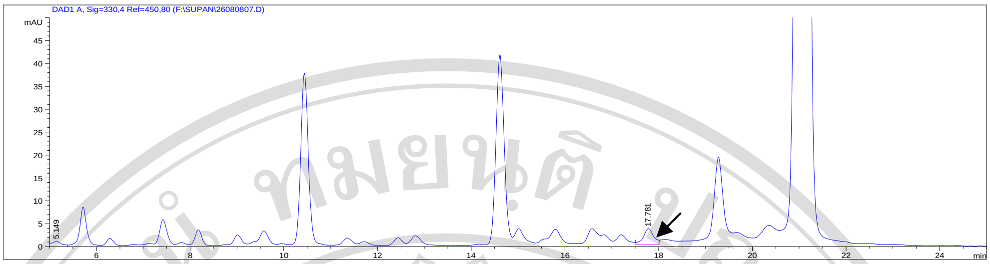


Figure D 3.9.1 Chromatograms of young leaves containing **tryptophan**

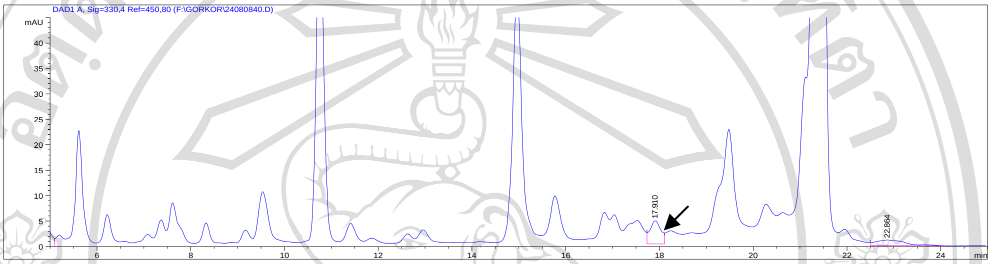


Figure D 3.9.2 Chromatograms of germinated rice grains containing tryptophan

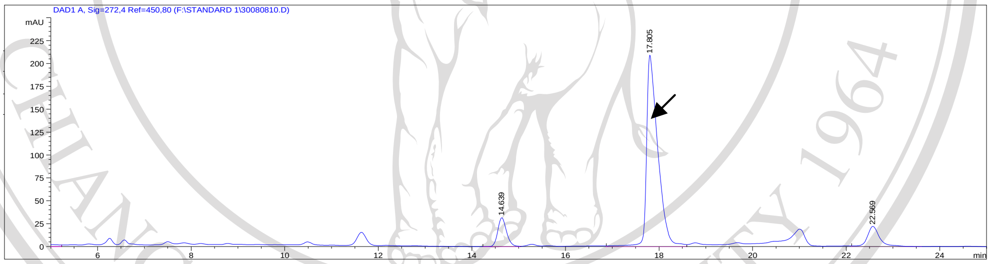


Figure D 3.9.3 Chromatograms of standard tryptophan

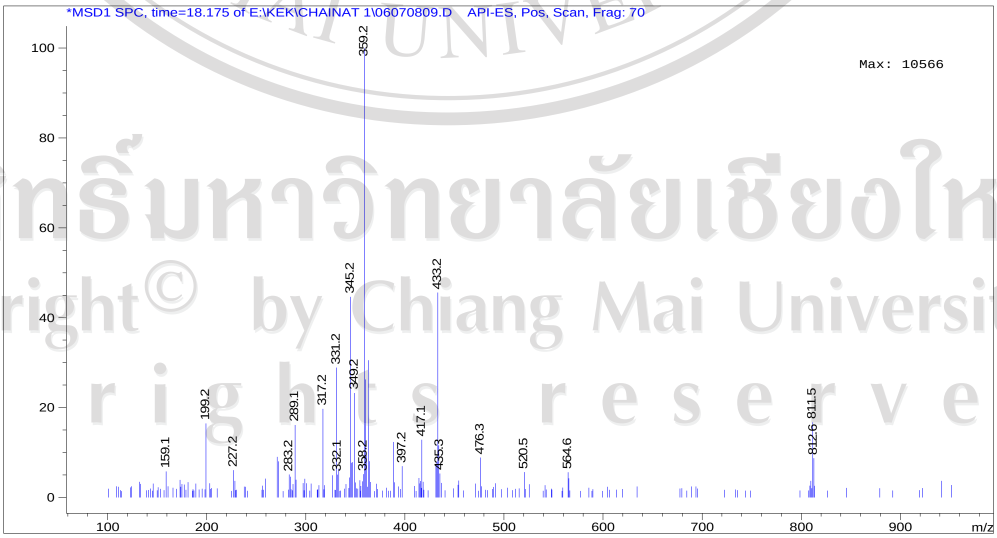


Figure D 3.9.4 Mass spectrum of sample containing tryptophan

3.10 Isoleucine

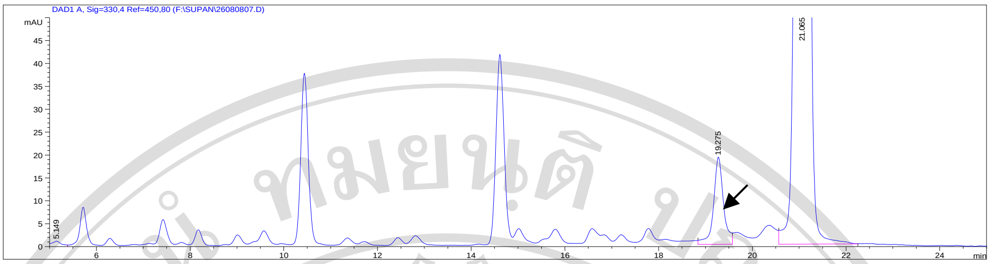


Figure D 3.10.1 Chromatograms of young leaves containing isoleucine

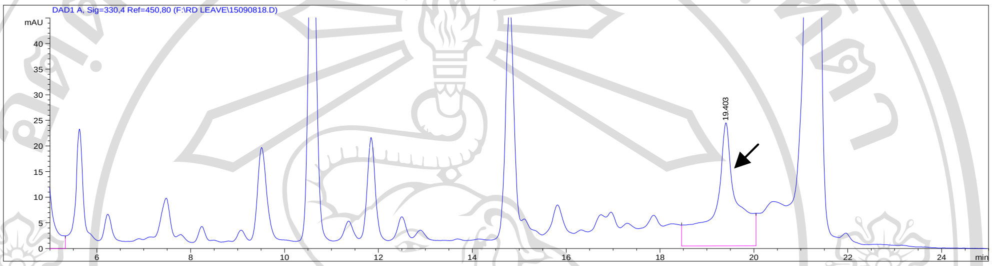


Figure D 3.10.2 Chromatograms of germinated rice grains containing isoleucine

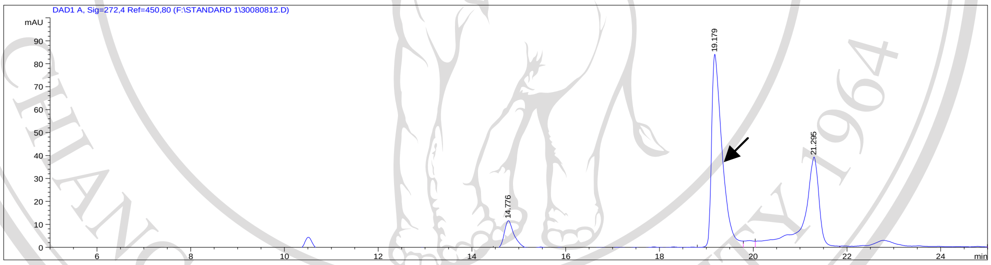


Figure D 3.10.3 Chromatograms of standard of isoleucine

Figure D 3.10.4 Mass spectrum of sample containing isoleucine

3.11 Leucine

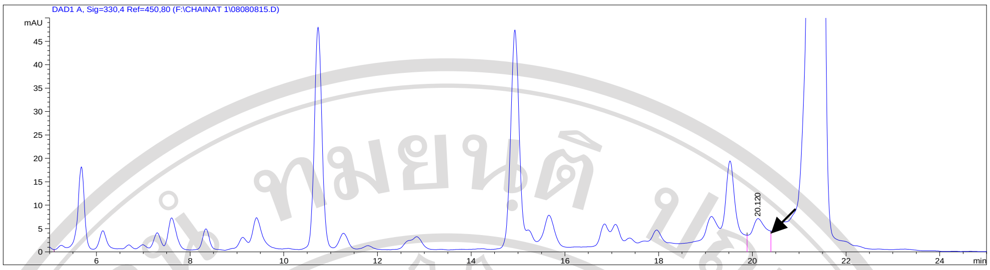


Figure D 3.11.1 Chromatograms of young leaves containing leucine

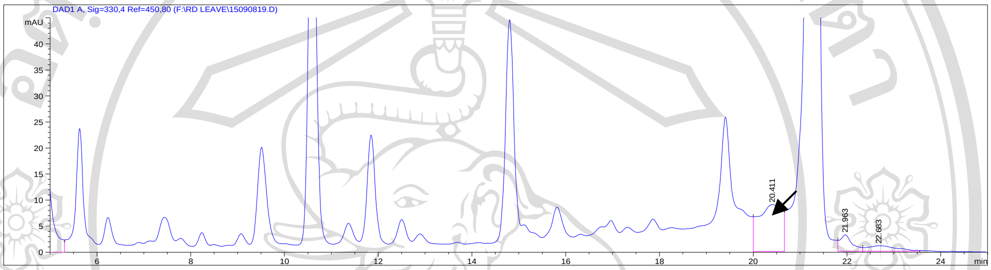


Figure D 3.11.2 Chromatograms of germinated rice grains containing leucine

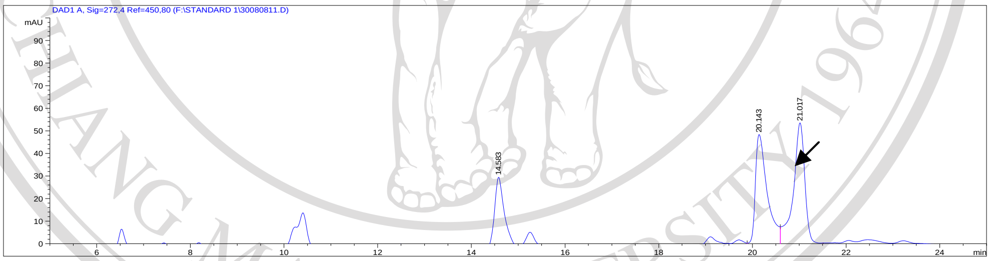


Figure D 3.11.3 Chromatograms of standard of leucine

Figure D 3.11.4 Mass spectrum of sample containing leucine

APPENDIX E

Mass spectrum, PMF and amino acid sequencing

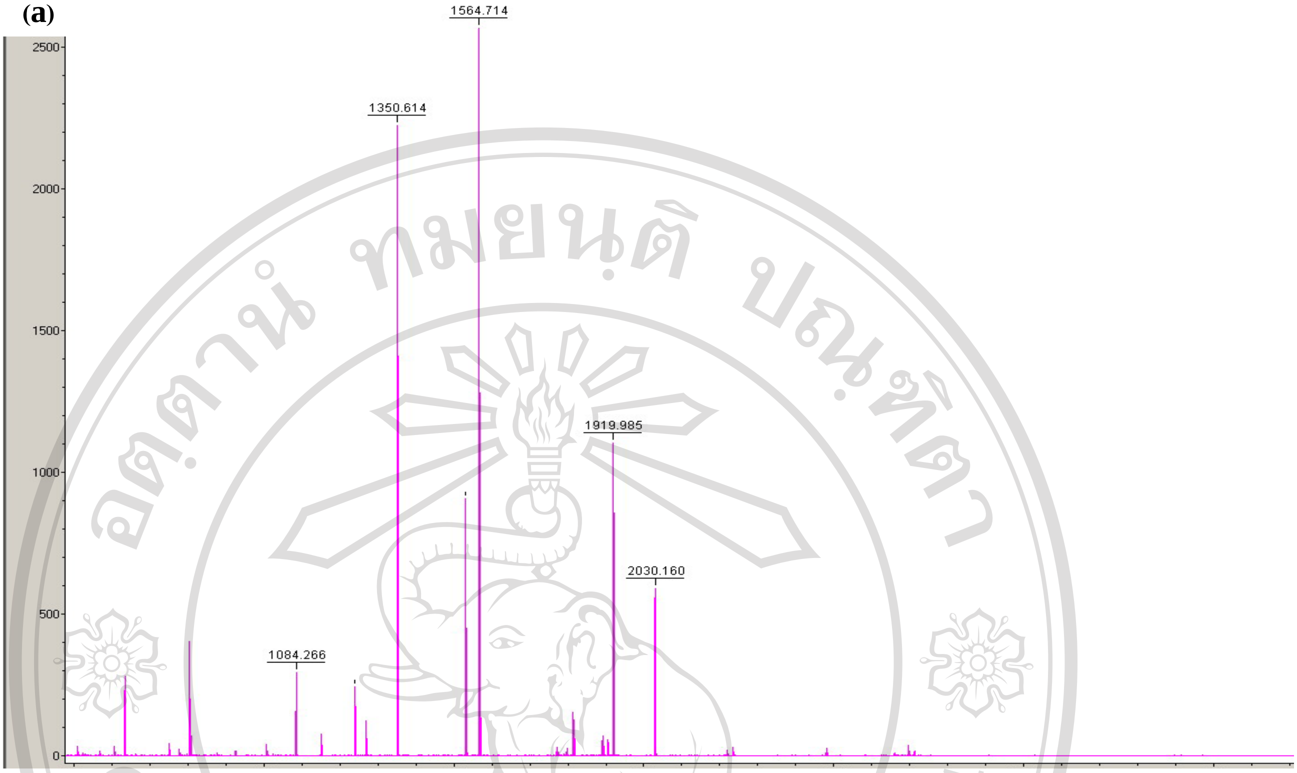
Selected protein spots (Spot C1, C2 and C3) were analyzed by MALDI-ToF MS instrument to determine the name and functions of interested protein . Mass spectrum and peptide mass finger print (PMF) were obtained from this instrument. PMF were used for searching in NCBI and Swissport database to determine closely PMF in database to PMF in experiment. Figure D.1-D.3 showed the mass spectrum, PMF and amino acid sequencing of protein spot C 1, C 2 and C3, respectively.

(**a**) Mass spectrum of protein ; y axis = signal intensity of fragment ion and X axis = m/z value

(**b**) PMF which obtained from Mass spectrum

(**c**) Amino acid sequencing of protein database that showed the highest similarity score when compare with analyzed protein; Red bold = Matched amino acids from database.

(a)

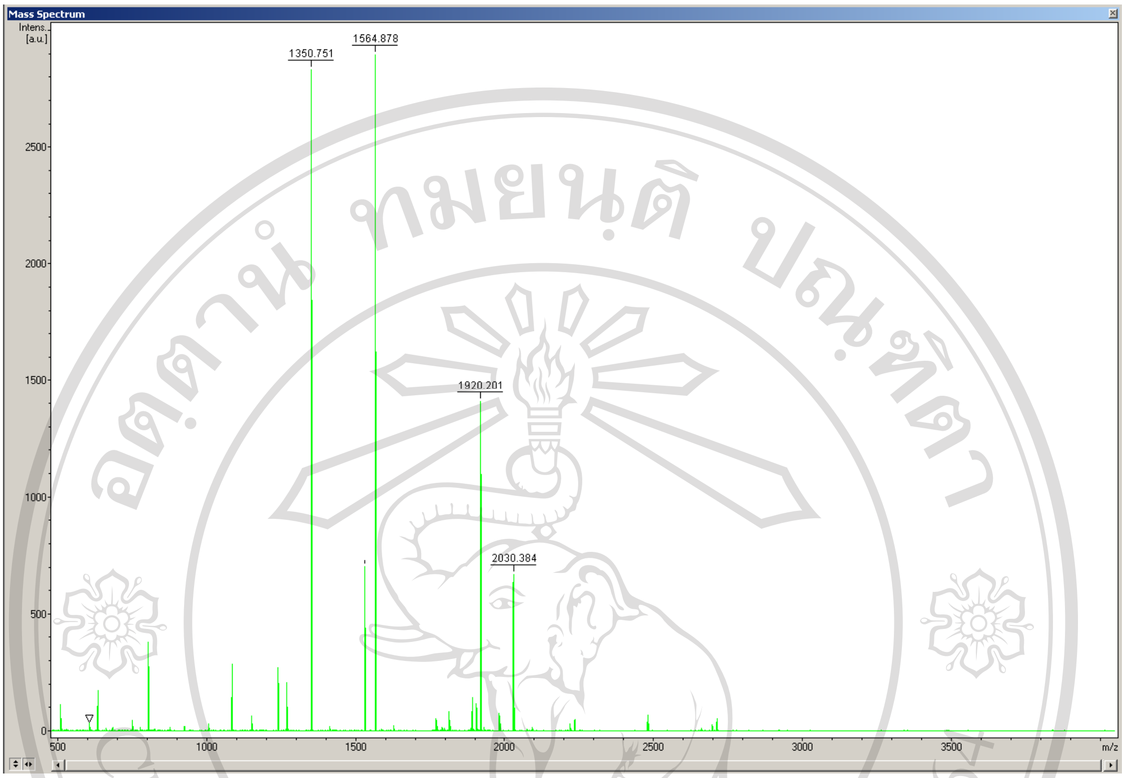


(b) Matched peptides shown in **Bold Red**

```
1   MAKKKTSSSM ARSQLAALLI SLCFLSLASN AVGWSRRGER EEEDERRRHG
51  GEGGRPYHFG EESFRHWTRT RHGRFSVLER FPDEQVVGAA VGGYRVAVLE
101 AAPRAFLQPS HYDADEVFYV KEGEGVIVLL REGRKESFCV REGDAMVIPA
151 GAIVYSANTH SSKWFRVVML LNPVSTPGHF EEYFPVGGDR PESFFSAFSD
201 DVLQAAFNTR REELEKVFER QREGGEITTA PEEQIRELSK SCSRGGGGGS
251 GSEWEIKPSS LTGKSPYFSN NHGKLFELTG DECRHLKKLD LQIGLANITR
301 GSMIAPNYNT RATKLAVVLQ GSGYFEMACP HVSGGGSSER REREREHGRR
351 REEEQGEEEH GERGEKARRY HKVRAQVREG SVIVIPASHP ATIVASEGES
401 LAVVCFFVGA NHDEKVFLAG RNSPLRQLDD PAKKLVFGGS AAREADRVLA
451 AQPEQILLRG PHGRGSVSDM
```

Figure E.1 Spot number C1; hypothetical protein OsI_13867 [*Oryza sativa* Indica Group]Function:0045735 - nutrient reservoir activity.

(**b**)



(**b**) Matched peptides shown in **Bold Red**

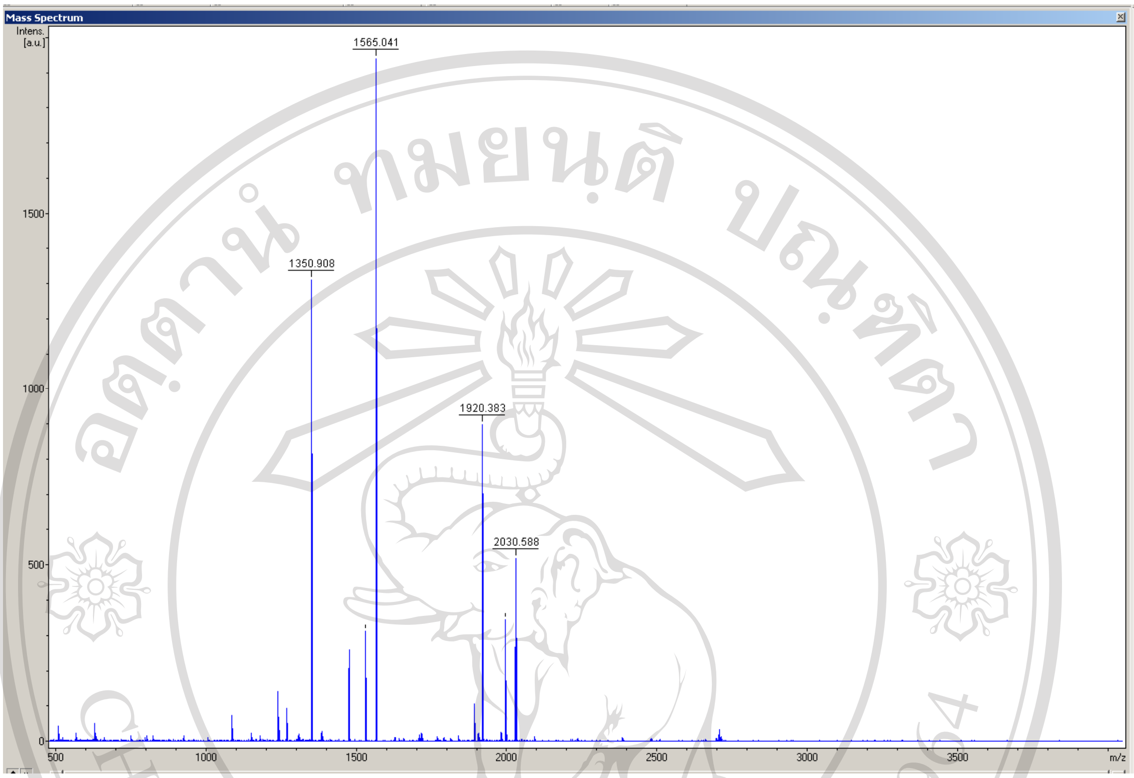
```
  1 MAKKKTSSSM ARSQLAALLI SLCFLSLASN AVGWSRRGER EEEDERRRHG
 51 GEGGRPYHFG EESFRHWTRT RHGRFSVLER FPDEQVVGAA VGGYRVAVLE
101 AAPRAFLQPS HYDADEVFYV KEGEGVIVLL REGRKESFCV REGDAMVIPA
151 GAIVYSANTH SSKWFRVVML LNPVSTPGHF EEYFPVGGDR PESFFSAFSD
201 DVLQAAFNTR REELEKVFER QREGGEITTA PEEQIRELSK SCSRGGGGGS
251 GSEWEIKPSS LTGKSPYFSN NHGKLFELTG DECRHLKKLD LQIGLANITR
301 GSMIAPNYNT RATKLAVVLQ GSGYFEMACP HVSGGGSSER REREREHGRR
351 REEEQGEEEH GERGEKARRY HKVRAQVREG SVIVIPASHP ATIVASEGES
401 LAVVCFFVGA NHDEKVFLAG RNSPLRQLDD PAKKLVFGGS AAREADRVLA
451 AQPEQILLRG PHGRGSVSDM
```

Matched (bold red) peptides: HG (49–50), GEGGRPYHFG, EESFR, FPDEQVVGAA, VGGYR, AFLQPS, HYDADEVFYV, K (121), EGGEITTA, PEEQIR, VLA (448–450), AQPEQILLR.

Figure E.2 Spot number C2; hypothetical protein OsI_13867 [*Oryza sativa* Indica Group]

Function: nutrient reservoir activity

(c)



(b) Matched peptides shown in **Bold Red**

```
  1 MAKKKTSSSM ARSQLAALLI SLCFLSLASN AVGWSRRGER EEEDERRRHG
 51 GEGGRPYHFG EESFRHWTRT RHGRFSVLER FPDEQVVGAA VGGYRVAVLE
101 AAPRAFLQPS HYDADEVFYV KEGEGVIVLL REGRKESFCV REGDAMVIPA
151 GAIVYSANTH SSKWFRVVML LNPVSTPGHF EEYFPVGGDR PESFFSAFSD
201 DVLQAAFNTR REELEKVFER QREGGEITTA PEEQIRELSK SCSRGGGGGS
251 GSEWEIKPSS LTGKSPYFSN NHGKLFELTG DECRHLKKLD LQIGLANITR
301 GSMIAPNYNT RATKLAVVLQ GSGYFEMACP HVSGGGSSER RERerehgrr
351 REEEQGEEEH GERGEKARRY HKVRAQVREG SVIVIPASHP ATIVASEGES
401 LAVVCFFVGA NHDEKVFLAG RNSPLRQLDD PAKKLVFGGS AAREADRVLA
451 AQPEQILLRG PHGRGSVSDM
```

Figure E.3 Spot number C3; hypothetical protein OsI_13867 [*Oryza sativa* Indica Group], globulin 2 Function: Storage protein in seed

APPENDIX F

Comparison of GABA and amino acids content between rice cultivars

Table F.1 Rice cultivars categories group based on their GABA and amino acids contents in rice grains

Compounds		**Rice Cultivars**		
GABA	**Lowest value**	**Low value**	**Medium value**	**Highest value**
0 days	Not different *(p>0.05)*			
5 days	PL2, SP1, RD6	RD6, CN1, PL2, KDML 105, PT1		
10 days	SP1, PL2, PT1	PL2, PT1, RD6	PT1, RD6, KDML105, CN1	RD6, KDML105,CN1, SPT1
15 days	Not different *(p>0.05)*			
20 days	Not different *(p>0.05)*			
25 days	Not different*(p>0.05)*			
30 days	PT1, PL2, SPT1, RD6	PL2, SPT1, RD6, CN1, SP1	CN1, SP1, KDML105	
Arginine				
0 days	KDML105, RD6, PL2, SPT1	RD6, PL2, SPT1, PT1, CN1	PT1, CN1, SP1	
5 days	Not different *(p>0.05)*			
10 days	PL2, SP1, KDML105, CN1	SP1, KDML105, CN1, PT1	KDML105, CN1, PT1, SPT1	PT1, SPT1, PL2
15 days	Not different *(p>0.05)*			
20 days	SP1, CN1, PL2, KDML105, SPT1, PT1	RD6		
25 days	PL2, SP1, CN1, PT1,SPT1	SP1, CN1, PT1, SPT1	RD6	
30 days	PL2, CN1, SPT1, PT1, RD6, SP1	KDML105		
Asparagine				
0 days	Not different *(p>0.05)*			
5 days	PL2	RD6, SP1, PT1, KDML105, CN1	SP1, PT1, KDML105, CN1, SPT1	
10 days	PL2	CN1	SP1, PT1, KDML105	KDML105, RD6, SPT1
15 days	PL2, CN1,	CN1, SP1, PT1	SP1, PT1, SPT1, RD6	SPT1, RD6, KDML105
20 days	PL2	SP1, RD6, SPT1, PT1, CN1	RD6, SPT1, PT1, CN1, KDML105	
25 days	PL2, SP1, CN1	SP1, CN1, PT1,SPT1, RD6	SP1,SPT1, KDML105,PT1	
30 days	PL2, PT1	PT1, CN1	CN1, RD6, SPT1, SP1	SPT1,SP1, KDML105

Table F.1 (continued) Rice cultivars categories group based on their GABA and amino acids contents in rice grains

Compounds	**Rice Cultivars group**			
	Lowest value	**Low value**	**Medium value**	**Highest value**
Serine				
0 days	SPT1,PL2,RD6	PL2,RD6,PT1	PT1,SP1, KDML105,CN1	
5 days	Not different ($p>0.05$)			
10 days	Not different ($p>0.05$))			
15 days	Not different ($p>0.05$))			
20 days	SPT1, PL2,SP1	PL2,SP1,PT1	SP1,PT1,CN1,KDML105, RD6	
25 days	SPT1, PL2	PL2,PT1,SP1,CN1, KDML105	PT1,SP1,CN1,KDML105, RD6	
30 days	SPT1,PT1,PL2	PL2,SP1,CN1, KDML105,RD6		
Glycine				
0 days	PL2,SP1,RD6	PT1, SPT1, CN1, KDML105		
5 days	PL2	SP1, RD6	RD6,SPT1	SPT1,CN1,PT1
10 days	PL2	SP1,PT1,CN1	PT1,CN1, KDML105, RD6	CN1, KDML105,RD6, SPT1
15 days	PL2,CN1	CN1,PT1,SPT1,SP3	PT1,SPT1,SP3, RD6	RD6, KDML105
20 days	Not different ($p>0.05$)			
25 days	PL2	CN1,SP1,PT1	PT1,SPT1	RD6
Threonine				
0 days	Not different ($p>0.05$)			
5 days	Not different ($p>0.05$)			
10 days	CN1,RD6,PL2,PT1	RD6,PL2,PT1,SP1	PT1,SP1,SPT1, KDML105	
15 days	RD6,CN1,PL2	CN1,PL2,PT1,SP1	PT1,SP1,SPT1	SPT1, KDML105
20 days	Not different ($p>0.05$)			
25 days	RD6,CN1,SP1,PL2,PT1	CN1,SP1,PL2,PT1, KDML105	PL2,PT1, KDML105,SPT1	
30 days	Not different ($p>0.05$)			

Table F.1 (continued) Rice cultivars categories group base on their GABA and amino acids contents in rice grains

Compounds	Rice Cultivars group			
Glutamic acid	**Lowest value**	**Low value**	**Medium value**	**Highest value**
0 days	RD6,PL2	PL2,SP1,PT1,SPT1	KDML105	CN1
5 days	RD6,PT1,PL2,SP1,SPT1	PT1,PL2,SP1, KDML105	CN1	
10 days	RD6,PL2,SP3, KDML105, SPT1, PT1	CN1		
15 days	PL2, RD6,SP1,PT1,SPT1	SP1,PT1,SPT1, KDML105	KDML105	
20 days	RD6, PL2,SP1,PT1, KDML105	CN1		
25 days	Not different *(p>0.05)*			
30 days	RD6	PL2,SPT1,PT1, SP1	SPT1,PT1,SP1, KDML105	KDML105,CN1
Alanine				
0 days	Not different *(p>0.05)*			
5 days	SP1,PL2,RD6,CN1,PT1	RD6,CN1,PT1,SPT1	CN1,PT1,SPT1, KDML105	
10 days	Not different *(p>0.05)*			
15 days	Not different *(p>0.05)*			
20 days	Not different *(p>0.05)*			
25 days	Not different *(p>0.05)*			
30 days	Not different *(p>0.05)*			
Tyrosine				
0 days	PL2,SP1	SP1,CN1,PT1	CN1,PT1,SPT1, KDML105	RD6
5 days	Not different *(p>0.05)*			
10 days	PL2,RD6,SP1,CN1,PT1, KDML105	SPT1		
15 days	PL2,SP1, CN1,PT1	KDML105,RD6,SPT1		
20 days	Not different *(p>0.05)*			
25 days	PL2,CN1,SP1,SPT1,PT1	CN1,SP1,SPT1,PT1, KDML105	KDML105,RD6	
30 days	SP1,PT1,PL2,SPT1,CN1	SPT1,CN1, KDML105	KDML105,RD6	

Table F.1 (continued) Rice cultivars categories group base on their GABA and amino acids contents in rice grains

Compounds	**Rice Cultivars group**			
Valine	**Lowest value**	**Low value**	**Medium value**	**Highest value**
0 days	PT1, SP1,RD6,SPT1	RD6,SPT1, KDML105	SPT1, KDML105,CN1	PL2
5 days	SP1,RD6,PT1,CN1	RD6,PT1,CN1, KDML105	KDML105,SPT1	PL2
10 days	SP1,CN1,PT1, KDML105,RD6,SPT1	PL2		
15 days	SP1,CN1,PT1, KDML105,RD6,SPT1	PL2		
20 days	SP1,PT1, KDML105,RD6,SPT1,PL2	CN1		
25 days	SP1,CN1,PT1, KDML105,RD6,SPT1	PL2		
30 days	SP1,CN1,PT1, KDML105,RD6,SPT1	PL2		
Tryptophan				
0 days	SP1	RD6	KDML105,PL2	SPT1, CN1,PT1
5 days	SP1,PL2	PL2, KDML105,CN1,SPT1,PT1	CN1,PT1,SPT1,RD6	
10 days	PT1,PL2,SP1, KDML105	KDML105,CN1	CN1,RD6,SPT1	
15 days	SP1,PT1, KDML105	PT1, KDML105,RD6,SPT1,CN1		
20 days	SP1,PL2,PT1 KDML105,CN1	PL2,PT1, KDML105, CN1,SPT1	RD6	
25 days	PT1,PL2	PL2,PT1,CN1,SPT1, KDML105	CN1, SPT1,KDML105,RD6	
30 days	Not different *(p>0.05)*			
Isoleucine				
0 days	Not different *(p>0.05)*			
5 days	Not different *(p>0.05)*			
10 days	PL2, SP1,CN1	SP1,CN1,PT1, KDML105,RD6	CN1,PT1,KDML105,RD6,SPT1	
15 days	SP1,CN1,KDML105,RD6	CN1,KDML105,RD6,SP1,PT1	KDML105,RD6,SP1,PT1,SPT1	
20 days	Not different *(p>0.05)*			
25 days	Not different *(p>0.05)*			
30 days	PL2,CN1	PT1,RD6,SP1, KDML105,PT1		
Leucine				
0 days	RD6,SP1	PL2	SPT1	PT1,CN1,KDML105
10 days	PL2,SP1,CN1,PT1	SP1,CN1,PT1, KDML105	RD6,SPT1	
15 days	PL2,SP1,CN1	SP1,PT1,RD6	KDML105,SPT1	
20 days	CN1,PL2,SP1,RD6	PL2,PT1,RD6, KDML105, PT1	SPT1	
25 days	Not different *(p>0.05)*			
30 days	CN1,PL2,RD6,PT1,SPT1	PT1,SPT1, KDML105,SP1		

Table F.2 Rice cultivars categories group base on their GABA and amino acids contents in rice leaves

Compounds	**Rice Cultivars**			
GABA	**Lowest value**	**Low value**	**Medium value**	**Highest value**
5 days	Not different ($p>0.05$)			
10 days	SPT1,SP1,CN1	RD6,PL2,PT1	KDML105, PT1	
15 days	Not different ($p>0.05$)			
20 days	Not different ($p>0.05$)			
25 days	Not different ($p>0.05$)			
30 days	SPT1,RD6, KDML105,PT1	SPT1,PL2,PT1	SPT1,PL2,CN1,SP1	
Arginine				
5 days	Not different ($p>0.05$)			
10 days	SP1,CN1	CN1,PL2,PT1, KDML105	KDML105,SPT1	
15 days	Not different ($p>0.05$)			
20 days	Not different ($p>0.05$)			
25 days	Not different ($p>0.05$)			
30 days	RD6, KDML105,PT1,SPT1	PT1,SPT1,PL2,CN1	PL2,CN1,SP1	
Asparagine				
5 days	Not different ($p>0.05$)			
10 days	Not different ($p>0.05$)			
15 days	Not different ($p>0.05$)			
20 days	Not different ($p>0.05$)			
25 days	Not different ($p>0.05$)			
30 days	KDML105,RD6	RD6,PT1,PL2	SP1,SPT1,CN1	
Serine				
5 days	Not different ($p>0.05$)			
10 days	PL2,CN1	SP1,PT1,RD2, KDML105	SPT1	
15 days	PL2,CN1,SP1	CN1, SP1,PT1,SPT1	PT1,RD6,SPT1	RD6,SPT1, KDML105
20 days	Not different ($p>0.05$)			
25 days	Not different ($p>0.05$)			
30 days	Not different ($p>0.05$)			

Table F.2 (continued) Rice cultivars categories group base on their GABA and amino acids contents in rice leaves

Compounds	Rice Cultivars			
Glycine	**Lowest value**	**Low value**	**Medium value**	**Highest value**
5 days	Not different *(p>0.05)*			
10 days	CN1, SP1,RD6,PL2	SP1,RD6,PL2, KDML105	RD6,PL2, KDML105,SPT1	PT1, KDML105
15 days	Not different *(p>0.05)*			
20 days	Not different *(p>0.05)*			
25 days	Not different *(p>0.05)*			
30 days	Not different *(p>0.05)*			
Threonine				
5 days	Not different *(p>0.05)*			
10 days	SP1,PL2	PL2,PT1, KDML105,CN1	PT1, KDML105,CN1,RD6,SPT1	
15 days	SP1,PT1,SPT1	PT1,SPT1,RD6, KDML105,CN1	RD6, KDML105,CN1,PL2	
20 days	Not different *(p>0.05)*			
25 days	Not different *(p>0.05)*			
30 days	Not different *(p>0.05)*			
Glutamic acid				
5 days	Not different *(p>0.05)*			
10 days	RD6,PL2,PT1,CN1	PL2,PT1,CN1,SP1	SP1, KDML105, SPT1	
15 days	RD6, CN1, PT1, KDML105,PL2	PL1	SPT1	
20 days	RD2,PL2,CN1,PT1,KDML105	SP1	SPT1	
25 days	Not different *(p>0.05)*			
30 days	PL2,RD6,PT1,CN1,SPT1	RD2, PT1,CN1,SPT1,SP1	SP1, KDML105	
Alanine				
5 days	Not different *(p>0.05)*			
10 days	SP1,PL2	PL2, KDML105	KDML105,RD6,SPT1	PT1,CN1
15 days	SP1,PL2,RD6,KDML105,SPT1	SPT1, CN1	CN1,PT1	
20 days	Not different *(p>0.05)*			
25 days	Not different *(p>0.05)*			
30 days	Not different *(p>0.05)*			

Table F.2 (continued) Rice cultivars categories group base on their GABA and amino acids contents in rice leaves

Compounds	**Rice Cultivars**			
Tyrosine	**Lowest value**	**Low value**	**Medium value**	**Highest value**
5 days	Not different *(p>0.05)*			
10 days	Not different *(p>0.05)*			
15 days	Not different *(p>0.05)*			
20 days	Not different *(p>0.05)*			
25 days	Not different *(p>0.05)*			
30 days	RD6,PL2,SPT1,CN1	PL2, SPT1,CN1, KDML105	SPT1,CN1, KDML105,PT1	SP1
Valine				
5 days	Not different *(p>0.05)*			
10 days	Not different *(p>0.05)*			
15 days	Not different *(p>0.05)*			
20 days	Not different *(p>0.05)*			
25 days	Not different *(p>0.05)*			
30 days	Not different *(p>0.05)*			
Tryptophan				
5 days	Not different *(p>0.05)*			
10 days	SP1,PT1	PT1,CN1,PL2	CN1,PL2,SPT1, KDML105	SPT1, KDML105,RD6
15 days	SP1,PL2	PL2,PT1,RD6	PT1,RD6, KDML105,SPT1,CN1	
20 days	No difference (*p>0.05)*			
25 days	SPT1	SP1,PL2	PL2,CN1,RD6	CN1,RD6,PT1,KDML105
30 days	No difference (*p>0.05)*			
leucine				
5 days	No difference			
10 days	No difference (*p>0.05)*	PT1,CN1,PL2	PL2,CN1,SPT1,KDML105	SPT1, KDML105,RD6
15 days	No difference (*p>0.05)*	PL2,PT1,RD6	PT1,RD6, KDML105,SPT1,CN1	
20 days	No difference (*p>0.05)*			
25 days	No difference (*p>0.05)*	SP1,PL2	PL2,CN1,PT1	CN1,RD6,PT1,KDML105
30 days	No difference (*p>0.05)*			

Table F.2 (continued) Rice cultivars categories group base on their GABA and amino acids contents in rice leaves

Compounds	Rice Cultivars			
5 days	No difference (*p*>0.05)			
10 days	KDML105,RD6,PT1	RD6,PT1,SP1	SP1,CN1	CN1,PL2,SPT1
15 days	No difference (*p*>0.05)			
20 days	No difference (*p*>0.05)			
25 days	No difference (*p*>0.05)			
30 days	KDML105,PT1	PT1,PL2,RD6	PL2,RD6,SP1	CN1,SPT1

Appendix G

GABA and amino acids contents

Table G.1 GABA contents in germinated rice grain and young leave of rice cultivars

Cultivars/germination days		GABA content (mg/g)	
SPT1		**Grain**	**Young Leaves**
	control	0.38 ± 0.00	-
	5	0.70 ± 0.13	-
	10	1.03 ± 0.03	0.42 ±0.46
	15	1.24 ± 0.20	0.63 ±0.23
	20	1.60 ± 0.88	1.77 ±0.29
	25	1.54 ± 0.36	2.28 ±0.27
	30	1.38 ±	1.39 ±1.09
RD 6			
	control	0.22±0.15	-
	5	0.60±0.08	-
	10	0.86±0.07	0.67±0.98
	15	0.81±0.01	0.98±0.34
	20	0.75±0.07	1.65 ±0.42
	25	1.16±0.98	1.73 ±0.09
	30	1.47±0.15	2.21 ±0.73
SP 1			
	control	0.34±0.05	-
	5	0.36±0.17	-
	10	0.49±0.19	0.56 ±0.47
	15	0.65±0.52	0.75 ±0.54
	20	1.10±0.29	3.91 ±0.62
	25	1.47±0.79	1.98 ±0.56
	30	1.73±0.17	2.09 ±0.64
PL 2			
	control	0.19±0.00	-
	72	0.33±0.11	-
	5	0.58±0.035	0.63±0.60
	10	0.58±0.14	1.02±0.18
	15	0.94±0.297	1.45 ±0.18
	20	1.25±0.11	1.85 ±0.55
	30	0.92±	3.16 ±0.33
KDML 105			
	control	0.50 ±0.06	
	72	0.72±0.12	
	5	0.92±0.28	1.02 ±
	10	1.28±0.28	1.19 ±0.18
	15	1.24±0.27	2.31 ±0.11
	20	1.42±0.24	2.17 ±0.35
	30	1.41±0.93	2.50 ±0.06

Table G.1 (continued) GABA contents in germinated rice grain and young leave of seven selected cultivars

Cultivars/germination days	GABA content (mg/g)	
	Grain	**Young Leaves**
PT 1		
control	0.39 ±0.06	-
72	0.73 ±0.15	-
5	0.71 ±0.14	0.81 ±0.41
10	1.09 ±0.20	1.07 ±0.23
15	1.35 ±0.17	1.50 ±0.15
20	1.59 ±0.57	1.33 ±0.24
30	0.98 ±	2.12 ±0.17
CN 1		
control	0.30 ±0.25	
72	0.67 ±0.17	
5	0.92 ±0.14	0.61 ±0.45
10	1.13 ±0.36	0.71 ±0.23
15	1.76 ±1.06	1.47 ±0.36
20	2.01 ±0.37	1.57 ±0.90
30	1.69 ±0.34	2.85 ±0.82

Table G.2 Amino acids content of germinated rice grain at different germination days of seven selected cultivars

Cultivars/ germination days	Amino acids content (mg/g)											
SPT1	**Arg**	**Asp**	**Ser**	**Gly**	**Glu**	**Tyr**	**Ala**	**Val**	**Thr**	**Tryp**	**Iso**	**Leu**
0	3.22	7.44	0.58	2.12	2.19	0.89	1.54	0.39	0.87	0.65	1.32	1.54
5	3.29	8.23	0.63	2.35	2.20	1.39	2.69	0.86	0.98	0.85	3.38	2.76
10	4.71	9.10	1.26	2.59	2.61	2.19	5.12	2.34	1.45	1.79	8.82	6.93
15	5.93	9.05	1.36	2.88	2.82	2.52	5.43	2.54	1.52	1.87	10.47	7.28
20	7.43	6.80	1.26	3.04	2.97	2.54	6.54	2.71	1.69	1.89	10.39	7.87
25	6.15	9.32	1.28	3.15	2.97	1.73	5.40	2.97	2.09	1.77	11.20	7.21
30	4.57	8.79	0.96	2.99	3.13	1.63	5.10	2.20	1.78	1.58	10.00	6.41
RD 6												
0	3.03	3.33	1.08	2.56	0.60	0.73	0.32	0.34	0.34	0.31	0.77	0.96
5	3.20	4.55	1.69	0.80	0.95	0.85	1.53	0.39	0.31	0.53	2.45	2.02
10	4.90	8.53	2.77	2.49	1.32	2.18	3.45	1.43	0.67	1.09	6.58	6.23
15	6.23	9.62	3.21	1.89	1.35	1.92	5.86	1.15	0.52	1.49	6.23	4.49
20	10.25	6.65	3.16	4.39	0.28	3.39	7.13	1.87	0.93	1.50	5.13	4.99
25	9.76	10.52	4.11	2.32	0.41	3.24	13.24	2.95	0.69	2.90	8.63	8.46
30	5.08	8.05	4.00	5.55	0.36	4.53	12.97	1.19	0.96	2.55	8.57	4.57
SP1												
0	3.88	6.83	1.97	0.89	1.64	0.56	0.74	0.19	0.80	0.34	1.89	0.98
5	3.21	5.27	1.87	1.46	1.68	0.71	1.09	0.28	0.82	0.45	2.17	1.40
10	3.77	6.68	2.26	1.76	1.91	0.88	1.97	0.53	1.17	0.69	4.16	2.30
15	4.56	6.49	2.33	2.80	2.08	1.15	3.24	0.96	1.11	0.91	6.48	3.27
20	4.36	5.59	2.33	2.55	2.00	0.97	2.26	0.92	1.18	1.54	7.43	4.01
25	4.29	7.27	2.89	2.34	2.42	1.13	2.83	1.30	1.01	2.52	9.49	5.88
30	6.03	9.04	3.29	2.66	3.75	1.59	3.53	1.72	1.25	1.39	9.45	8.34

Table G.2 (continued) Amino acids content of germinated rice grain at different germination days of seven selected cultivars

Cultivars/ germination days	**Amino acids content (mg/g)**											
	Arg	**Asp**	**Ser**	**Gly**	**Glu**	**Tyr**	**Ala**	**Val**	**Thr**	**Tryp**	**Iso**	**Leu**
PL2												
0	3.03	1.92	0.98	0.65	1.32	0.35	0.51	0.67	0.43	0.58	0.86	1.13
5	3.09	1.53	1.13	0.86	1.64	0.57	1.20	0.75	0.56	0.68	0.99	1.77
10	3.37	1.59	1.26	0.95	1.87	0.79	1.80	0.84	0.76	0.67	1.36	1.49
15	3.69	2.69	1.79	1.55	1.32	0.93	2.33	0.94	0.90	0.79	4.83	2.31
20	5.02	2.63	2.41	2.06	1.93	1.24	3.65	1.56	1.20	0.96	6.03	3.82
25	3.53	4.94	1.91	1.37	3.02	1.43	2.75	1.67	1.56	1.30	2.08	5.29
30	3.44	5.11	1.80	1.41	2.40	1.41	3.83	1.90	1.69	1.49	5.57	4.45
KDML105												
0	2.75	3.51	1.97	2.12	1.85	0.96	0.68	0.58	1.24	0.56	1.56	2.34
5	2.94	5.88	2.07	2.69	2.25	1.10	3.12	0.66	1.18	0.77	2.93	2.92
10	3.82	7.06	2.81	2.89	3.34	1.47	3.48	1.42	1.58	0.95	6.37	3.96
15	5.41	10.20	3.08	2.70	2.99	2.16	4.99	1.99	1.62	1.45	5.85	6.80
20	6.24	8.07	3.10	3.66	3.09	2.54	4.72	1.71	1.80	1.29	7.56	5.41
25	9.41	11.74	3.50	3.58	5.03	2.69	4.82	2.39	1.81	1.79	8.07	6.97
30	7.83	10.08	3.97	3.41	5.34	2.80	5.70	2.57	1.89	1.82	9.65	7.80

Table G.2 (continued) Amino acids content of germinated rice grain at different germination days of seven selected cultivars

Cultivars/ germination days	Amino acids content (mg/g)											
	Arg	**Asp**	**Ser**	**Gly**	**Thr**	**Glu**	**Ala**	**Tyr**	**Val**	**Tryp**	**Iso**	**Leu**
PT1												
0	3.37	6.88	1.49		0.72	1.83	0.94		0.17		1.18	1.68
5	3.54	5.80	1.51	2.10	0.63	1.60	2.03	0.82	0.50	0.78	2.79	3.61
10	4.43	6.81	2.48	2.38	0.83	3.86	3.31	1.29	1.11	0.93	5.50	4.09
15	4.92	6.81	2.04	2.16	0.95	1.97	2.98	1.24	1.17	0.66	7.10	5.78
20	6.31	7.36	2.56	2.49	1.51	2.98	4.38	1.62	1.64	1.32	7.75	5.84
25	5.07	9.02	2.85	2.59	1.79	3.35	3.78	1.73	1.65	1.24	7.94	6.21
30	4.27	5.88	1.23	2.50			3.00	1.21	1.21	0.49	9.36	5.01
CN1												
0	3.44	4.42	2.16	2.52	0.62	4.74	2.23	0.79	0.67	0.76	1.84	1.78
5	3.39	6.43	2.05	2.29	1.25	8.79	1.81	0.80	0.53	0.81	2.67	2.00
10	3.86	7.72	2.37	2.29	0.47	5.81	2.82	1.18	1.05	1.39	4.99	2.65
15	4.06	5.28	2.47	2.33	0.69	5.39	2.86	1.20	1.20	1.95	3.92	2.53
20	5.01	7.63	3.03	2.48	0.81	9.04	3.90	1.62	1.47	1.77	5.61	3.19
25	4.81	7.38	3.15	2.13	0.92	6.87	3.55	1.58	1.75	1.73	5.05	4.42
30	3.59	7.38	3.61	2.16	0.65	6.61	4.79	1.81	0.66	1.68	3.66	4.37

Table G.3 Amino acids content of young leaves at different germination days of seven selected cultivar

Cultivars/ germination days	Amino acids content (mg/g)											
	Arg	**Asp**	**Ser**	**Gly**	**Thr**	**Glu**	**Ala**	**Tyr**	**Val**	**Tryp**	**Iso**	**Leu**
SPT1												
5	5.42	7.41	1.85	1.08	0.86	1.34	2.45	0.71	0.65	0.98	4.54	3.21
10	7.41	9.67	2.86	2.49	1.24	4.56	4.41	1.07	1.25	1.57	7.52	4.40
15	9.37	16.42	3.38	7.35	1.40	7.76	6.91	2.87	2.08	2.12	11.35	8.19
20	14.50	24.34	4.83	6.32	1.80	7.89	6.83	2.03	3.74	3.11	13.93	12.88
25	10.84	10.95	3.94	3.97	1.80	6.71	5.21	1.91	2.95	6.34	13.14	2.57
30	8.78	9.94	4.17	3.64	2.73	3.69	8.86	1.90	3.05	5.76	22.88	10.50
RD6												
5	3.56	12.56	1.13	0.98	0.76	0.98	2.34	0.87	0.97	1.12	2.67	2.34
10	5.76	14.35	1.89	1.53	1.12	1.18	4.12	1.23	1.85	1.67	3.56	5.67
15	7.87	29.96	2.67	1.67	1.65	2.06	5.33	1.71	1.84	1.95	7.72	7.18
20	11.58	44.89	3.72	1.12	1.52	2.41	5.56	1.96	2.30	1.94	10.44	8.95
25	9.32	41.36	5.30	2.38	2.45	4.34	9.27	2.62	2.99	4.08	15.27	12.51
30	4.82	18.62	1.60	2.15	1.42	2.79	3.43	0.98	1.01	1.32	11.22	5.06
SP1												
5	3.34	3.57	0.88	0.35	0.26	2.19	0.87	0.79	0.35	0.54	1.81	0.96
10	4.47	6.45	1.57	1.86	0.35	3.45	1.13	0.99	0.46	0.93	4.76	1.18
15	6.55	7.58	1.74	1.72	0.43	4.68	2.60	1.12	0.74	1.87	5.33	2.75
20	16.52	23.91	3.10	3.55	1.33	5.71	5.63	3.42	2.99	2.69	8.19	8.13
25	12.58	36.89	3.00	3.26	1.63	3.71	3.89	4.22	3.41	3.47	10.73	7.38
30	15.41	56.67	3.35	2.54	1.68	4.66	9.52	5.32	3.41	3.19	12.23	10.67
PL2												
5	3.27	10.49	0.48	1.40	0.94	1.98	1.90	0.97	0.87	0.90	6.09	1.48
10	5.87	11.26	0.67	2.09	1.12	2.07	2.29	1.07	1.14	1.24	6.99	3.79
15	8.85	13.05	0.95	2.44	2.34	3.03	3.11	1.14	1.54	1.44	7.03	4.92
20	15.94	24.24	1.74	5.64	1.90	2.49	3.80	2.79	2.41	1.85	7.97	6.48
25	19.16	51.38	2.50	4.26	2.36	3.08	5.65	1.38	3.89	3.03	12.07	9.39

Table G.3 (continued) Amino acids content of young leaves at different germination days of seven selected cultivars

Cultivars/ germination days	Amino acids content (mg/g)											
	Arg	**Asp**	**Ser**	**Gly**	**Thr**	**Glu**	**Ala**	**Tyr**	**Val**	**Tryp**	**Iso**	**Leu**
PL2												
30	13.12	36.10	1.94	4.45	2.37	2.61	4.65	1.61	2.89	5.50	10.74	6.55
KDML105												
5	4.32	8.17	1.54	1.23	0.56	2.13	1.27	1.34	2.08	1.08	1.43	2.87
10	6.76	13.52	2.02	2.23	0.94	4.08	3.27	4.34	3.10	2.11	2.47	4.87
15	9.81	34.44	3.85	3.04	1.68	2.99	6.48	5.61	2.48	2.04	6.73	8.13
20	13.40	51.63	3.88	2.99	1.47	3.48	5.75	8.92	5.21	0.90	5.29	9.50
25	11.02	43.36	4.10	3.65	2.06	3.97	6.49	3.56	7.15	4.48	16.02	15.83
30	6.03	9.40	2.05	2.77	1.78	6.44	2.99	3.00	3.88	5.33	3.20	5.31
PT1												
5	3.56	8.95	0.96	1.10	0.32	0.92	10.43	0.98	1.16	0.98	1.84	1.32
10	6.29	12.65	1.78	3.26	0.89	2.18	13.51	1.26	1.79	1.85	3.92	2.69
15	9.34	23.33	2.37	3.19	0.92	3.18	16.84	2.57	2.03	2.11	5.33	5.98
20	11.05	28.52	2.76	2.90	1.49	3.43	12.38	2.41	2.97	2.34	7.34	8.33
25	14.80	65.35	4.85	3.86	2.92	3.70	5.79	3.58	5.40	5.05	17.62	12.69
30	7.75	35.62	3.32	3.75	1.71	4.28	6.40	3.31	2.41	2.76	5.24	7.49
CN1												
5	3.09	3.90	0.78	1.00	0.94	1.76	1.14	0.67	0.62	0.65	3.57	1.97
10	5.07	5.75	0.84	1.55	1.09	2.36	7.55	0.84	0.80	0.87	6.14	3.29
15	12.24	30.77	1.70	1.76	1.88	2.50	12.46	1.33	1.73	3.69	8.74	8.46
20	9.57	15.65	2.37	4.70	2.72	2.69	4.88	2.13	3.75	6.22	12.36	10.06
25	5.50	17.43	3.74	2.60	2.44	3.30	5.56	2.15	3.65	8.26	15.52	12.06
30	13.15	71.25	2.90	2.79	2.64	3.61	7.08	2.17	1.99	2.70	19.30	11.73

CURRICULUM VITAE

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1999 Sawan-anan Wittaya School, Sukhothai, Thailand

2002 B.Sc (Biochemistry and biochemical technology), Chiang Mai University, Thailand

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INTERNATIONAL & NATIONAL MEETING:

1. Panatda Jannoey and Griangsak Chairote**.** *Oral presentation* in the title "Change of gamma-amino butyric acid (GABA) and GAD protein in germinating rice" The 2nd International Meeting for Development of International Network for Food safety Technology in Southest Asia. 22-23 September 2009, CMU-KAGAWA symposium 2009, Thailand.
2. Panatda Jannoey and Griangsak Chairote. *Oral presentation* in the title "GABA Accumulations in Rice during Germination" Pure and Applied Chemistry International Conference 2009 (PACCON 2009). 14-16 January 2009 , Phitsanulok, 65000, Thailand.
3. Panatda Jannoey and Griangsak Chairote. *Poster presentation* in the title " Study of the glutamic acid and gamma-amino butyric acid (GABA) contents during germination of rice grain" National Conference of the commission on higher education 2008. 5-7 September 2008, Pattaya, Thailand.

4. Panatda Jannoey and Griangsak Chairote *Poster presentation* in the title "Preliminary screening of gramma-aminobutyric acid (GABA) producing microorganisms" International Conference of the Center for innovation in Chemistry: Postgraduate Education and Research Program in Chemistry, PERCH-CIC. Theme: Chemistry for Innovation, 2007. 6-9 May 2007, Pattaya, Thailand
5. Panatda Jannoey and Griangsak Chairote *Poster presentation* in the title. Lactic acid fermentation by *Peddiococcus* sp. and *Lactobacillus* sp. and recovery by solvent extraction. 28 th Congress on science and technology, Thailand, 2005.
6. Panatda Jannoey and Griangsak Chairote *Poster presentation* in the title. Volatile compounds of NHAM (Traditional THAI fermented food) made from porks and other materials. 29th Congress on science and technology, Thailand, 2004.
7. Panatda Jannoey and Griangsak Chairote. *Poster presentation* in the title. Comparison of volatile compounds in fermented sausage (NHAM). 16 th Annual Meeting of Thai society for innovative biotechnology, The opportunity for kitchen of the world, Thailand, 2003.
8. Panatda Jannoey and Griangsak Chairote. *Poster presentation* in the title. Retention of NHAM flavor. 31th Congress on science and technology, Thailand, 2002.

PUBLICATIONS;

1. Panatda Jannoey , Hataichanoke Niamsup, Saisamorn Lumyong , Toshisada Suzuki , Takeshi Katayama and Griangsak Chairote. Comparison of gamma-aminobutyric acid (GABA) production in Thai rice grains. *World Journal of Microbiology and Biotechnology*. XX-XX-2009, *Inpress.*
2. Panatda Jannoey , Hataichanoke Niamsup, Saisamorn Lumyong, Mika Nomura, Shideyugi Tajima and Griangsak Chairote. γ-Aminobutyric Acid (GABA) Accumulations in Rice During Germination . *Chiang Mai J. Sci.* XX-XX-2009, *Inpress.*

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1. Center for innovation in Chemistry: Postgraduate Education and Research Program in Chemistry (PERCH-CIC) Scholarship, 2006-2009, Thailand.
2. Frontier Research Network for the Ph.D. Program for Thai Doctoral Degree from the Office of the Higher Education Commission, Scholarship, 2006-2009, Thailand.
3. The Exchange Program for East Asian Young Researchers Scholarship (JSPS) Scholarship of Japan.