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THESIS

PROTEOMICS ANALYSIS OF SALINITY RESPONDING PROTEINS IN CELL SUSPENSION OF RICE (*Oryza sativa* L. subsp. indica)

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A Thesis Submitted in Partial Fulfillment of
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Salt stress is a major abiotic factor that limits crop productivity worldwide, especially rice which is a main food and an important exporting product of Thailand. Previous studies reported that in the affected paddy field, NaCl leads to changes of the physiological and biochemical processes of rice to overcome the deleterious effect. In this study, the activity of various enzymes and salt responding proteins during NaCl in rice cell suspension of salt-tolerant Pokkali, HJ and Thai Jasmine rice KDML105 salt-sensitive IR29 and PT1 and were determined by enzyme assay and GeLC-MS analysis.

The salt stress responding enzyme activity via β-glucosidase, peroxidase and ATPase were studied for 48 h. The different patterns of enzyme activity in the salt-tolerant and salt-sensitive rice were found. The relative activity of peroxidase and ATPase except β-glucosidase showed the significantly up-regulation in salt stress. In proteomic analysis, 43 differential expressed proteins were clustered in 13 clusters depending on their expression profile during salt stress. These proteins were identified and involved in transcription and translation processes, protein metabolism, sugar metabolism, energy metabolism, secondary metabolite synthesis, hormone synthesis, glutathione metabolism, binding and transporting protein, cell defense and response protein, signal transduction and skeleton and cell organization. Among 43 proteins, peroxidases, GST and NADH dehydrogenase subunit 5 were expressed as salt stress protein. The expression of peroxidase, GST and NADH dehydrogenase subunit 5 were found in the salt-tolerant PK, HJ and KDML 105 but not found in the salt-sensitive IR29 and PT1.

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I gratefully thanks to plant physiology and biochemistry laboratory for providing rice seeds cultivar KDML105 and HJ. Miss Piyaporn Kongkeaw, Miss Thapanee Samphumphoung and others in the plant laboratory were acknowledged for teaching tissue culture technique when I started my lab. I am heartfelt thanks to my friends in the department of botany and my lovely sister, Miss Yada Boonsorn for their kindness help. Especially, I would like to give appreciation to my family, my parents, my sister and brother for their special care and love and continuing encouragements.

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Sawanya Charoenlappanit

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LIST OF ABBREVIATIONS

p-ATPase	=	plasma membrane H^+ adenosine triphosphate
v-ATPase	=	vacuolar H^+ adenosine triphosphate
DW	=	dry weight
FW	=	fresh weight
h	=	hour
HJ	=	Hawm Jan
KDML105	=	Thai Jasmine rice or Khao Dawk Mali 105
mg	=	milligram
min	=	minute
ml	=	milliliter
mM	=	millimolar
MS	=	Murashige and Skooge
Picloram	=	4-amino-3,5,6,trichloro-picolinic acid
PK	=	Pokkali
POD	=	peroxidase
PT1	=	Pathumthani 1
ROS	=	reactive oxygen species
SH	=	Schenk and Hildebrandt
U	=	unit of enzyme
μg	=	microgram
μl	=	microliter
μM	=	micromolar

PROTEOMICS ANALYSIS OF SALINITY RESPONDING PROTEINS IN CELL SUSPENSION OF RICE (*Oryza sativa* L. subsp. indica)

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's most important crops and a primary staple food for more than a third of the world's population. However, most paddy field is usually affected by salt stress which results in rice productivity decreasing. The conventional breeding technologies and proper management strategies play a vital role in rice improvement but they have little success in improving the desirable results. So the new methods and technologies are important alternative to answer the deleterious problem. In salt stress, rice has developed different physiological and biochemical strategies to adapt or tolerate these stress conditions by regulation of ion and compartmentalization, biosynthesis of compatible solutes and anti-oxidative enzymes and synthesis of plant hormones. However these responses are controlled by some protein expression genes (Parida and Das, 2005). Therefore, to understand the function of this mechanism in rice, its expression patterns in response to salt stress was investigated by proteomic analysis.

The changed of protein expression in rice has been reported that under salt stress, indicates that salt stress has a negative effect on rice photosynthesis, photorespiration, fatty acid synthesis, DNA processing, protein biosynthesis and etc. (Sobhanian *et al.*, 2011). The studies by proteomic analysis, rice also responds to salt stress by increasing the expression of proteins as superoxide dismutase, ascorbate peroxidase, catalase, ATPase, phenylalanine ammonia-lyase, aldolase, enoyl-ACP reductase which involved in defense, detoxification, and osmotic adjustment. So the studied salt responsive proteins in rice using a proteomic technique which is a powerful molecular tool for describing the complete proteome at the organelle, cell, organ, or tissue level. Moreover the studies of rice proteome have been reported in

leaves, root, seedling and callus but not only the suspension cell (Abbasi and Komatsu, 2004; Sobhanian *et al.*, 2011).

In this study, the cell suspension of the salt tolerant rice Pokkali and salt sensitive rice IR29, Thai jasmine rice KDML105, Pathumthani1 and Hawm Jan suspension cell was used as the plant model to study differential expressions of salt responsive proteins under sodium chloride stress.

OBJECTIVES

1. To get the efficient plant cell model for studying in salt responsive.
2. To study the protein expression in suspension cell of salt tolerant and salt sensitive rice by proteomic analysis techniques.

Hypothesis

Salt stress can differently induce the protein expression between the salt tolerant and salt sensitive rice suspension cells.

LITERATURE REVIEW

1. Salt Stress

1.1. Salinity definition

Saline soil or salt affected soil is defined as the salt-containing soils which was having high concentration of soluble salts to affect plant growth. Salt affected soil is caused by the excess salt accumulation. Salt concentration in a soil is measured in terms of its electrical conductivity, as described in the section below on measurements. The USDA Salinity Laboratory defines a saline soil as having an ECe of 4 dS/m or more. ECe is the electrical conductivity of the 'saturated paste extract', that is, of the solution extracted from a soil sample after being mixed with sufficient water to produce a saturated paste. However, many crops are affected by soil with an ECe less than 4dS/m. The moisture content of a drained soil at field capacity may be much lower than the water content of its saturated paste. Further, under dryland agriculture, the soil water content might drop to half of field capacity during the life of the crop. The actual salinity of a rain-fed field whose soil had an ECe of 4 dS/m could be 8-12 dS/m, this would severely limit yield of most crops.

Table 1 The comparisons of salt affects soils.

	pH	EC (dS/m)*	ESP**
Normal soil	6.5-7.2	<4	<15
Acid soil	<6.5	<4	<15
Saline soil	<8.5	>4	<15
Sodic soil	>8.5	<4	>15
Saline-sodic	<8.5	>4	>15

* electrical conductivity (EC) in decisiemen/meter (ds/m)

** exchangeable sodium percentage (ESP)

1.2. Salt-tolerant species and the criteria for salt stress tolerance

In general, plants have different degrees of salt tolerant traits which are divided into 4 types (Orcutt *et al.*, 2000).

1) Glycophytes are the plant whose growth and fitness decrease when exposed to any level of salinity greater than approximately 10 mM.

2) Halophytes or salt tolerant species means the species that tolerate higher salinity than glycophytes (up to approximately 50 mM) before a reduction in growth or fitness occurs.

3) Obligate halophytes are the species that do not attain their highest fitness at low soil salinity ((less than 10 mM). Moderately high soil salinity (50 mM) is required to attain maximum fitness.

4) Facultative halophytes are the species that attain a halophytic nature only after they experience moderate salinity in the soils or as an aerosol.

Mostly, halophytes can tolerate to salinity in seawater level or 500 mM sodium chloride while, ghycophytes commonly are sensitive to salinity greater than 50 mM sodium chloride. Rice was reported as a glycophyte that yields reduction of 50% when exposured to 40 mM sodium chloride (Orcutt and Nilsen, 2000). However, rice shows a wide range of salt tolerance ability. Among rice cultivars, the level of salt tolerance was divided into salt resistant rice as Pokkali, moderately salt tolerant rice as IR4595-4-1-13 and IR98884-54-3-1E-P1), and salt sensitive rice as IR29 (Gregorio *et al.*, 2002).

1.3. Stress and plant response

Salinity in the agricultural field is thus a severe constraint to crop growth and productivity in many regions, and the situation has become a global concern. It is estimated that 20% of irrigated land in the world is affected by salinity (Yamaguchi and Blumwald, 2005). Moreover, it has been predicted that increasing salinization in agricultural fields will reduce the land available for cultivation by 30% within the next 25 years, and up to 50% by the year 2050 (Wang *et al.*, 2003). Excess salt in soil or in solutions interferes with several physiological and biochemical processes, resulting in problems such as ion imbalance, mineral deficiency, osmotic stress, ion toxicity and oxidative stress; these conditions ultimately interact with several cellular components, including DNA, proteins, lipids, and pigments in plants (Zhu, 2002), impeding the growth and development of a vast majority of crops. The protection of crops against salinity induced damage has become a global challenge. High salinity (e.g., increased concentrations of sodium ion and chloride ion in the soil solution) causes osmotic and ionic stress (Hasegawa *et al.*, 2000). To protect from the detrimental effects of salt-stress, plants have evolved many biochemical and molecular mechanisms. The main biochemical strategies are induction of antioxidative enzymes, ion homeostasis and synthesis of compatible organic solutes.

1) Antioxidative defense system: during salinity induced oxidative stress, several cytotoxic reactive oxygen species (ROS) are continuously generated in the mitochondria, peroxisome and cytoplasm, which can destroy the normal metabolism through oxidative damage of lipids, proteins and nucleic acids (Apel and Hirt, 2004; Foyer and Noctor, 2005; Turkan and Demiral, 2009). ROS mainly comprises of superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH•) and singlet oxygen ($^1O_2$) as shown in Fig. 1. In the course of evolution, plant cells have developed the complex antioxidant defense system both enzymatic (SOD, APX, GPX, GR, CAT, etc.) and non-enzymatic (ascorbate, glutathione, tocopherol, carotenoids, flavonoids, etc.) to protect themselves against salt stress (Noctor and Foyer, 1998; Hossain *et al.*, 2007; Turkan and Demiral, 2009). In many studies differences have been found in levels of expression and activity of antioxidative enzymes; these

differences are sometimes associated with more tolerant genotypes, and sometimes with the more sensitive genotypes (Munns and Tester, 2008).

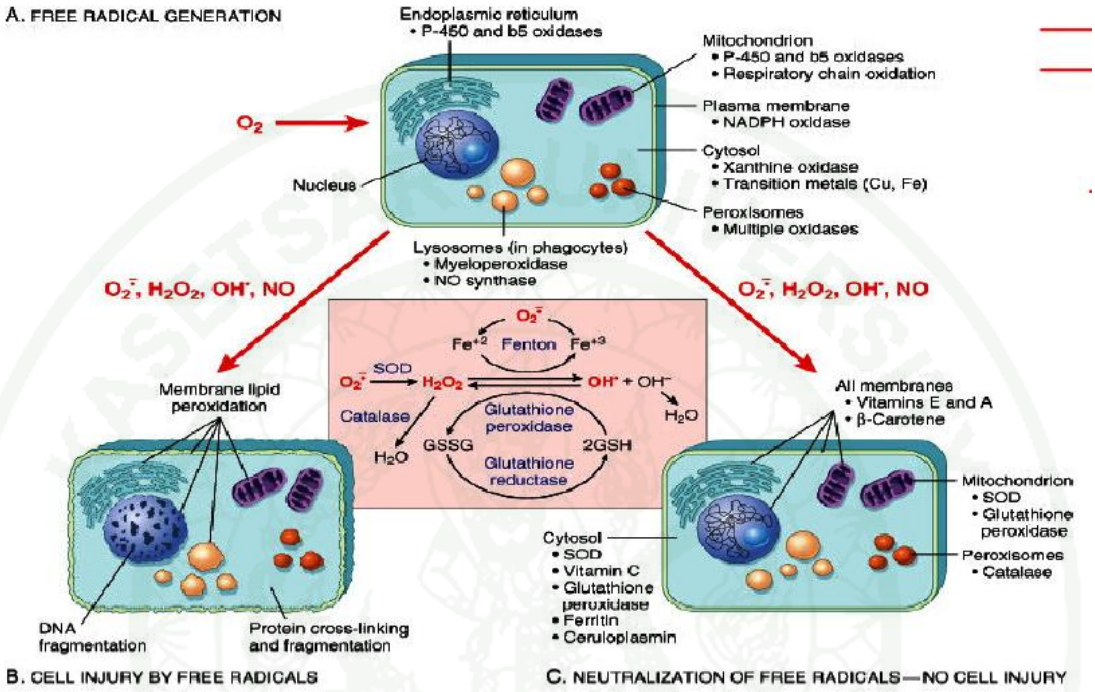

Figure 1 The reactive oxygen species (ROS) generation.

Source: Chabot *et al.* (1998)

2) Ion-homeostasis: salinity causes ion-specific stresses resulting from altered K^+/Na^+ ratios lead to be build-up in Na^+ and Cl^- concentrations. That is detrimental to plants. The alteration of ion ratios in plants is due to the influx of Na^+ through pathways that function in the acquisition of K^+. Maintenance of a high cytosolic K^+/Na^+ ratio is a key requirement for plant growth under high concentration of salt (Yamaguchi and Blumwald, 2005). Plants use different strategies to maintain a high K^+/Na^+ ratio in the cytosol, diminishing the entry of Na^+ ions into the cells, extrusion of Na^+ ions out of the cell and vacuolar compartmentation of Na^+ ions. Ion

exclusion mechanism can provide a degree of tolerance to relatively low concentrations of NaCl but will not work at high concentrations of salt, resulting in the inhibition of key metabolic processes and concomitant growth inhibition (Yamaguchi and Blumwald, 2005). Cytosolic K^+ homeostasis could be maintained by preventing NaCl induced K^+ leakage from the cell through the enhanced activity of H^+/ATPase. This could create the electron gradient favoring the ion transport process. Enhancement in proton pumping activity would furnish plasma membrane Na^+/H^+ antiporter with a driving force to expel Na^+ out of the cytoplasm into the apoplast and thus reducing cytosolic Na^+ load (Turkan and Demiral, 2009). The NHX-type antiporters, i.e. Na^+/H^+ located in tonoplast have been reported to increase salt-tolerance in many plant species by driving Na^+ accumulation in a vacuole (Apse *et al.*, 1999; Leidi *et al.*, 2010).

3) Accumulation of compatible solutes: salinity results from an excess of NaCl, the most common type of salt stress, the concentrations of sodium (Na^+) in the plant increase and concentrations of potassium (K^+) is reduced. This is called salt-specific or ion-excess effect of salinity (Kumar *et al.*, 2008). For overcoming salt stress, plants have evolved protective mechanisms that allow them to acclimatize. These mechanisms include osmotic adjustment. Under salt stress, plants restrict the uptake of salt and adjust their osmotic pressure by the synthesis of compatible organic solutes. Compatible solutes are low molecular weight, highly soluble compounds that are usually nontoxic at high cellular concentrations. These solutes include proline, sucrose, polyols, trehalose and quaternary ammonium compounds (QACs) such as glycine betaine, alaninebetaine, prolinebetaine, cholineO-sulfate, hydroxyprolinebetaine and pipecolatebetaine (Ashraf and Foolad, 2007). Proline, is one of the most studied compatible solutes playing a predominant role in protecting plants from osmotic stress (Tripathi and Gaur, 2004; Sumithra *et al.*, 2006; Ashraf and Foolad, 2007; BenAhmed *et al.*, 2008; Parida *et al.*, 2008; Rai *et al.*, 2010). Under salt stress, several functions are proposed for the accumulation of proline in tissues which include osmotic adjustment, carbon and nitrogen reserve for growth after stress recovery, detoxification of excess ammonia, stabilization of membranes, protecting photosynthetic activity and mitochondrial functions, and scavenging of free radicals

(Silveira *et al.*, 2003; KaviKishore *et al.*, 2005). However, the significance of proline accumulation in osmotic adjustment is still controversial and varies according to the species (Silveira *et al.*, 2003; Meloni *et al.*, 2004).

2. Rice

Rice is a member of the family Graminae or Poaceae, tribe Oryzeae. The genus *Oryza* contains approximately 22 species. Of which, 20 are wild species and two, *Oryza sativa* (Asian rice) and *Oryza glaberrima* Steud (African rice) are cultivated (Vaughan, 1994). Most common grown species throughout the world, *Oryza sativa* is differentiated into three subspecies in Asia based on geographic condition and amylase content as japonica, javanica and indica. *Oryza sativa* L. is more economically important than *Oryza glaberrima*, which is locally grown in West Africa (Grist, 1975). Among ecotype varieties, the indica rice is cultivated in wide regions and now rapid substituted other varieties because of the good growing and cooking properties. The indica rice has a high tillering capability. A clump is tall, so it exhibits considerably drought tolerance and resistance to insect pests and diseases. The grain is medium to long, with high amylose content, resulting in dry and fluffy cooked rice that shows little disintegration. Moreover, they are composed of aromatic flavor and highly valued. Most of the indica subspecies are grown in Indian, Southern China, South America and Thailand. Javanica subspecies are primarily grown in Indonesia, Malaysia and Philippines, located in the rice terraces of the Philippines and the mountainous regions of Madagascar. However, this subspecies are gradually disappeared due to the rapid spread of the modern indica subspecies which are high yield cooking quality and high adaptability to field condition (Christou, 1994).

Oryza sativa or rice uses the green leaf for photosynthesis and changes the macronutrient, micronutrient, water and carbon dioxide to starch for growth, development and produced the grain. The plant breeding used the grain from fertilization. The rice grain composes of endosperm and embryo that grown to seedling later.

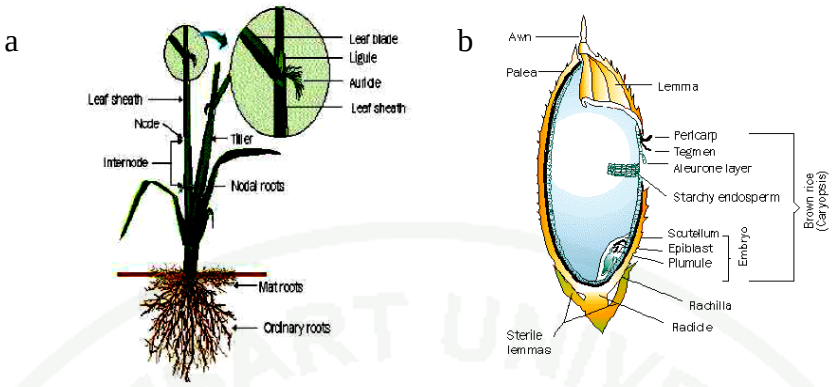

Figure 2 Morphology of the vegetative parts (a) and rice grain (b).

Source: Maclean *et al.* (2002)

Thai Jasmine rice or Khao Dawk Mali 105 (KDML 105) is the cultivated rice and give the average of yield 363 kg/rai. The quality of cultivar gave the color of the grain is white like the jasmine and good quality when cooking. KDML105 is resistant to the root-knot nematode but cannot resistant the blast, bacterial leaf blight or bacterial blight, yellow-orange leaf, rice ragged stun, brown planthopper, green rice leafhopper and rice stem borers.

Pathumthani1 (PT1) is the cultivated rice and gave the high yield average 650-774 kg/rai. The quality of cultivar is similar to the jasmine rice and good quality when cooking. The properties of PT1 are resistant to the brown planthopper, whitebacked planthopper, blast disease and bacterial blight.

Hawm Jan (HJ) is the rice which comes from the rice breeding 230 varieties physiology and biochemistry of salt-tolerant rice under the environmental control project. This rice capable tolerated at 342 mM NaCl and gave the yield average 516 kg/rai (Kirdmanee, 2008).

Pokkali (PK) is a salt-tolerant rice that comes from the experimental field which stress with the unsuitable environmental. The first successful in salt-tolerant selection in 1939 at Sri Lanka (Moeljopawiro and Ikahashi, 1981), PK is well known as a salt-tolerant donor in classical breeding and is commonly grown in the coastal area of Kerala, India. It is a traditional, tall, photoperiod sensitive rice cultivar that is susceptible to lodging and has a low tillering capacity with long, broad, dark, and droopy leaves. Moreover, the leaves senescence occurs quickly after flowering. The grain has pericarp and poor cooking quality (Kawasaki *et al.*, 2001; Gregorio *et al.*, 2002) showed that PK continued growing at a low photosynthetic rate after 7 days of salt stress, plant biomass approximately doubled. It was known that PK maintained water content in the shoot during a 6-week stress under the conditions of 150 mM NaCl. In contrast, salt sensitive IR29 showed a slightly slower response to the shock treatment, and the plants wilted irreversibly after 24 h. These results indicated that Pokkali achieved tolerance by rapidly expressing mechanisms for efficiently withstanding salt stress more than the salt-sensitive IR29.

Mostly, rice cultivated varieties are very sensitive plants to salt stress. Once, salt tolerant plants differ from salt sensitive by the low rate of Na^+ and Cl^- absorption through root, these ions move to the leaves, and are detoxified by compartmenting in vacuoles. Thus, salt tolerant ability is the capability to avoid the ion toxicity and prevent the increase of salt related-ion in cytoplasm or cell wall.

3. Suspension cell culture

Cell culture is the complex process by which cells are grown under controlled conditions. The cell suspension defines the single cell or the small cell aggregates from tissue culture technology in the liquid medium on the shaker. So, the suspension cell culture means the suspension cells which proliferate and complete growth cycle while suspended in the liquid medium. The suspension cell is induced from the proper tissue or plant cell that is the friable callus. For the reason, callus is the loose adhere plant cells which are easily to be aggregated to single cell. The useful of the suspension cell cultures are particularly suitable for the physiological, biochemical

and molecular studies of the process of somatic embryogenesis and its different stage induction development, maturation and conversion, the development of a genome and proteome analysis, the cellular metabolism studying and protoplast synthesis (Naill and Roberts, 2005; Iantcheva *et al.*, 2006). The cell culture and micro-propagation technology have made significant progress in the last two decades encompassing a wide variety of plant species. As an automation system becomes a more common feature in many laboratories, mass micro-propagation potentially can be automated to produce virtually unlimited quantities of somatic embryos in a mechanized, controlled environment. The cycle of embryogenic cell production through the suspension culture is the relatively short and cell mass can be proliferated quickly (Utomo *et al.*, 2008). That provides a rapid proliferation via the suspension culture. This method has been established in several plants such as *Arabidopsis thaliana* (Chevalier *et al.*, 2004), *Lilium formosanum* (Nakano *et al.*, 2000), *Elaeis guineesis* (Kanchanapoom and Tinnongjig, 2001), *Agapanthus praecox* (Suzuzi *et al.*, 2002), *Manihot esculenta* (Gonzalez *et al.*, 1998) and *Spartina alterniflora* (Utomo *et al.*, 2008).

4. Proteomic analysis

4.1. Proteome and proteomics

Proteins are an essential biomolecule that is necessary parts of organisms and participate in virtually every process within cell. For synthesis of protein, a succession of tRNA molecules charged with appropriate amino acids have to be brought together with a mRNA molecule and matched up by base-pairing through their anti-codons with each of its successive codons. The amino acids then have to be linked together to extend the growing protein chain, and the tRNAs, relieved of their burdens, have to be released. This whole complex of processes is carried out by a giant multimolecular machine, the ribosome, formed of two main chains of RNA, called ribosomal RNA (rRNA), and more than 50 different proteins. This molecular juggernaut latches onto the end of a mRNA molecule and then trundles along it, capturing loaded tRNA molecules and stitching together the amino acids they carry to form a new protein chain. So, proteome defines as the protein complement of the

genome. Each of cells contains all the information necessary to make a complete organism being. However, not all the genes are expressed in all the cells. Genes that code for enzymes essential to basic cellular functions are expressed in virtually all cells, whereas those with highly specialized functions are expressed only in specific cell types. Thereby, all cells express genes whose protein products provide essential functions and genes whose protein products provide unique cell-specific functions. Thus, every organism has one genome but many proteomes. The proteome in any cell represents some subset of all possible gene products. Any protein, though a product of a single gene, may exist in multiple forms that vary within a particular cell or between different cells. Indeed, most proteins exist in several modified forms. These modifications affect protein structure, localization, function, and turnover (Liebler, 2002). Therefore, proteomics is the study of the proteome. These "-omics" terms symbolize a redefinition of how we think about biology and the workings of living systems (Wilkins *et al.*, 1996). For above reasons, the studying protein content in cell is related the organelle and time that it changes in time and space.

In the recent past, extensive research has resulted in impressive achievements in genome and expressed sequence tag (EST) sequencing, yielding a wealth of information for many model organisms, including *Arabidopsis thaliana*, *Oryza sativa*, *Zea mays* and Medicago. However, genome sequence information alone is insufficient to reveal the facts concerning gene function, developmental/regulatory biology and the biochemical kinetics of life. To investigate these facts, more comprehensive approaches that include quantitative and qualitative analyses of gene expression products are necessary for the transcriptome, proteome and metabolome levels. Although transcriptome analysis using microarray and serial analysis of gene expression technologies are potential tools, mRNA and protein levels (Gygi *et al.*, 1999; Futcher *et al.*, 1999) cannot be correlated due to the inability of total mRNA to translate into protein. Whereas proteomics provides a more direct assessment of the biochemical processes of monitoring the actual proteins performing the signalling, enzymatic, regulatory and structural functions encoded by the genome and transcriptome.

4.2. Strategy for proteomic analysis

A general strategy for protein analysis that is utilized in proteomics is shown in Fig. 3. The workflow consists of several phases, each of which may be critical to the overall success of the analysis.

4.3. GeLC-MS/MS

GeLC-MS/MS is a powerful but simple approach for shotgun proteomic analyses. Samples are separated using 1-D SDS-PAGE and gel slices excised all the way down the track of the gel. These fractions are subsequently reduced and alkylated, and in gel digestion performed using a site-specific protease such as trypsin.

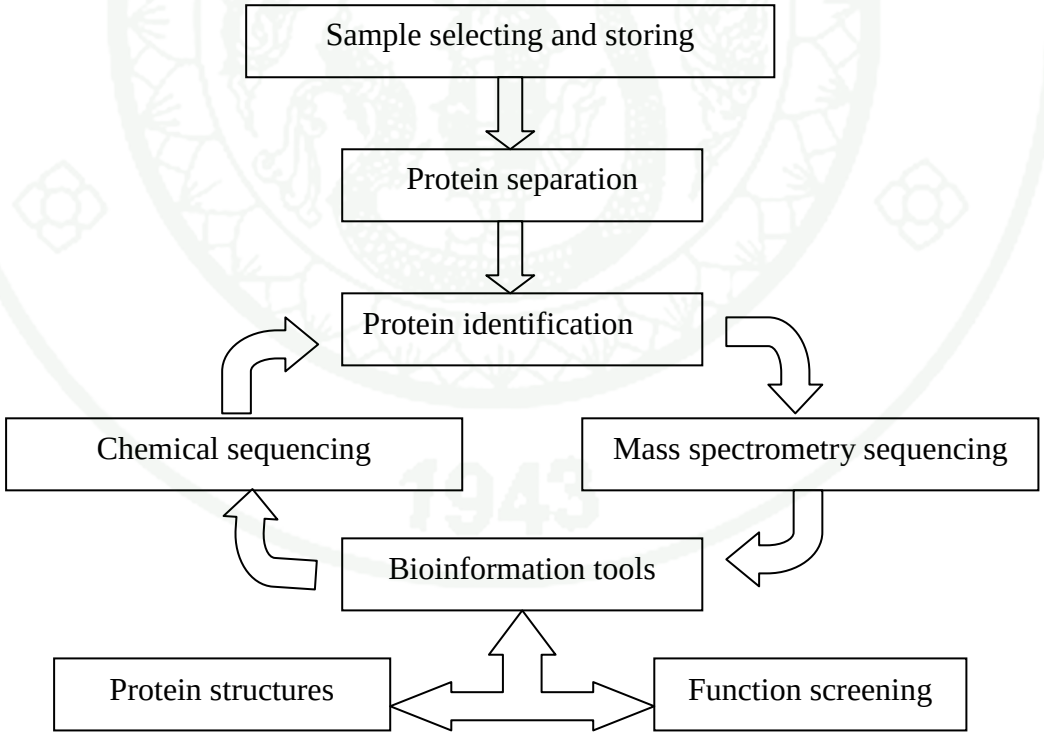

Figure 3 Schematic of the work flow in proteomic analysis

Source: Silberring (2008)

A nanoLC-MS/MS experiment is then performed to obtain peptide sequence information and hence identify the protein via using the database information. The steps involved in a GeLC/MS experiment are sample preparation, SDS-PAGE, segmentation, automated in-gel digestion, nanoLC-MS/MS, data analysis-including Mascot.

One dimension sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) is a simple and best way to estimate protein masses. This technique widely used in biochemistry and genetics and molecular biology to separate proteins according to their electrophoretic mobility (a function of length of polypeptide chain or molecular weight). SDS gel electrophoresis of samples have identical charge per unit mass due to binding of SDS results in fractionation by size (Liebler, 2002). SDS (sodium dodecyl sulfate) is a detergent that can dissolve hydrophobic molecules but also has a negative charge (sulfate) attached to it. Therefore, if a cell is incubated with SDS, the membranes will be dissolved, all the proteins will be solubilized by the detergent, plus all the proteins will be covered with many negative charges. So a protein that started out like the one shown in the top part of Fig. 4a) will be converted into the one shown in the bottom part of Fig. 4a). The result has two important features are all proteins retain only their primary structure , and all proteins have a large negative charge which means they will all migrate towards the positive pole when placed in an electric field (Fig. 4). All the proteins enter the gel small molecules can run through the polyacrylamide pore faster than big molecules.

After gel electrophoretic separation, protein must be visualized in some manner such as Coomassie Brilliant Blue staining, silver staining, amido black, copper and fast green staining, zinc salt, SDS and imidazole staining, fluorescence staining or isotope labeling (Table 2). In order that, the proper detection method is extremely important, because the proteomic approach often relies on measuring quantitative changes in expression level. The detection limit should be as low as possible with an optimal-to-noise ratio. The process should be easy and fast, nontoxic, cheap and MS compatible.

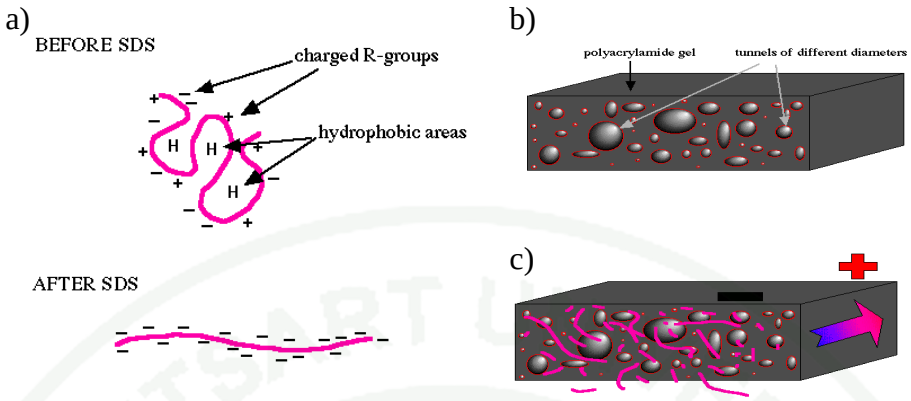

Figure 4 This cartoon depicts a protein (pink line) when it is incubated with the denaturing detergent SDS. The top portion shows a protein with negative and positive charges due to the charged R-groups in the protein. The large H represents hydrophobic domains where nonpolar R-groups have collected in an attempt to get away from the polar water that surrounds the protein. The lower shows that SDS can disrupt hydrophobic areas and coat proteins with many negative charges. The resulting protein has been denatured by SDS (reduced to its primary structure) and as a result has been linearized; a), A slab of polyacrylamide (dark gray) with tunnels (different sized red rings with shading to depict depth) exposed on the edge. Those are many different sizes of tunnels scattered randomly throughout the gel; b), A mixture of denatured proteins (pink lines of different lengths) beginning through a polyacrylamide gel (gray slab with tunnels). An electric filed is established with the positive pole (red plus) at the far end and the negative pole (black minus) at the closer end. Since all the proteins have strong negative charges, they will all move in the direction the arrow is pointing (run to be red); c).

Source: Davidson College. 2001

Table 2 Comparison of staining technique.

Staining method	Advantages	Disadvantages
Comassie	MS compatible Linearity range up to three orders of magnitude (colloidal solution) Low cost Good reproducibility	Detection limit 8 to 10 ng
Silver ammonia	Detection limit below 1 ng High reproducibility	Linearity range one order of magnitude Multistep protocol
Zinc-imidazol-SDS	MS compatible High sensitivity Detection limit 1 to 10 ng	Negative staining makes quantitative analysis impossible
Fluorescsnt dye	MS compatible High reproducibility Detection limit around 1 ng Reversible process Simple procedure Linearity range up to three orders of magnitude Possibility of PTMs' analysis	Requirement to use an ultraviolet or laser scanner
Isotope	High sensitivity Detection limit below 300 pg	Low reporducibility

A protein profiling strategy, variously referred to shotgun proteomics, multidimensional LC/MS/MS, or multidimensional protein identification technology (MudPIT), involves solution proteolysis of a complex mixture of proteins, followed by chromatographic separation of peptides prior to MS/MS sequencing (Bogdanov and Smith, 2005; McDonald and Yates, 2003). Often, protein separation and enrichment are carried out before digestion, for example, by protein chromatography or organelle purification. A variation of this approach separates proteins by SDS–PAGE, followed by in-gel digestion of proteins which comigrate in gel slices within narrow mass ranges, and subsequent multidimensional LC/MS/MS (Li *et al.*, 2003). Improved software in current mass spectrometers allows peptide sequencing by data-

dependent data acquisition, in which ions are automatically selected and fragmented by MS/MS, enabling thousands of spectra to be collected in a single reversed phase analysis (Link *et al.*, 1999; Gygi *et al.*, 1999; Washburn *et al.*, 2001). The viability of shotgun proteomics for global protein profiling was first shown in a study identifying more than 1400 proteins from *Saccharomyces cerevisiae*, including low abundance proteins (Washburn *et al.*, 2001). Recent studies have identified 1504 (25% of ORFs) from S. cerevisiae, 1910 proteins in *Deinococcus radiodurans* (61% of ORFs), 2415 proteins in *Plasmodium falciparum* (46% of ORFs), and 5130 proteins (15% of ORFs) in human erythroleukemia cells (Peng *et al.*, 2003; Lipton *et al.*, 2002; Florens *et al.*, 2002; Resing *et al.*, 2004).

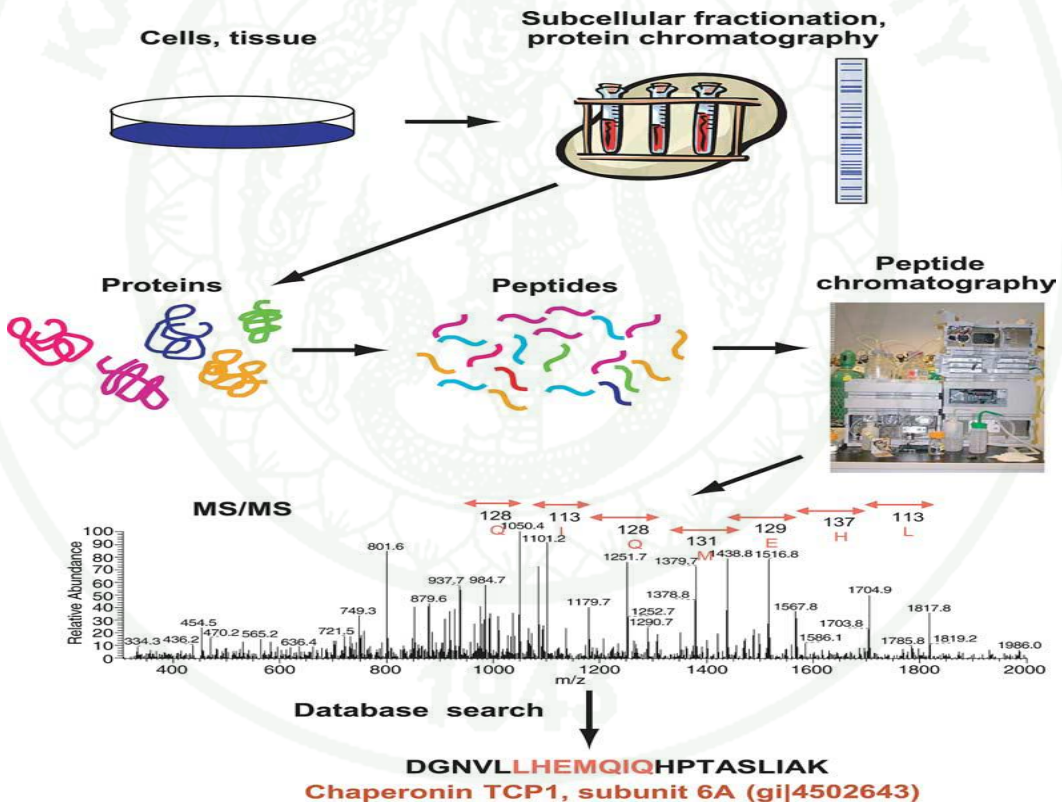

Figure 5 Protein profiling by shotgun proteomics. Complex mixtures of proteins are proteolyzed in solution, and resulting peptides are simplified by chromatographic separation prior to MS/MS sequencing.

Source: Resing and Ahn (2005)

4.4. Reviews of plant proteomics under the stress

The technology has been particularly useful in analyzing and comparing synthesis, turnover and modification of proteins during plant growth, development or response to environmental changes (Chen and Hamon, 2006). Comparative proteomics has been successfully applied for systematic scrutiny of proteins in several plant species under a wide range of abiotic challenges, including salt stress (Pandhal *et al.*, 2009; Jellouli *et al.*, 2008; Abbasi and Komatsu, 2004), drought (Selekdeh *et al.*, 2002; Hajheidari *et al.*, 2005; Alvarez *et al.*, 2008), high or low temperature (Majoul *et al.*, 2003; Gao *et al.*, 2009), ultraviolet radiation (Decker *et al.*, 2003), and heavy metals (Hu *et al.*, 2003). Proteomic applications provide a powerful tool for the study of plant response to salt stress (Zang and Komatsu, 2007; Dooki *et al.*, 2006; Pang *et al.*, 2010).

For previously study, the salt-stress proteins in plasma membranes of *Synechocystis* cells by proteomic analysis resulted in the identification of 109 proteins. The expression of twenty proteins was enhanced, and five proteins were reduced during salt stress. Seven of the salt enhanced proteins were periplasmic-binding proteins of ABC transporters. Within this group, the proteins that exhibited the highest expression enhancement included FutA1 (iron-binding protein) and Vipp1 (vesicle-inducing protein in plastids1), which have been suggested to be involved in protection of photosystem II (PSII) and in thylakoid membrane formation, respectively. While other proteins induced by salt-stress were regulatory proteins (Huang *et al.*, 2006).

In foxtail millet (*Setaria italica* L. cv. Prasad) seedlings responded to the salt stress, 175 protein spots reproducibly detected on 2D PAGE. Some were up-regulated, and few others were down-regulated at least at one time point. Mass spectrometry analysis allowed the identification of 29 differentially expressed proteins, including well-known salt responsive proteins indicated that the proteins are known to be involved in several processes, i.e., of signal transduction, photosynthesis,

cell wall biogenesis, stress related and several metabolisms like energy, lipid, nitrogen, carbohydrate and nucleotide metabolisms (Veeranagamallaiah *et al.*, 2008).

MATERIALS AND METHODS

1. Raw material

Seeds of rice (*Oryza sativa* L. subsp. indica) cultivars, Thai jasmine rice (KDML105), Pathumthani1 (PT1), Hawm Jan (HJ), Pokkali (PK) and IR29 (IR29) were obtained from the Pathumthani Rice Research Center (Rice Research Institute, Department of Agriculture, Ministry of Agriculture and Cooperative, Thailand).

2. Reagents

2.1. Reagents for plant material preparation

1) 4-amino-3,5,6,trichloro-picolinic acid (picloram)
2) Ammonium nitrate (NH_4NO_3)
3) Ammonium phosphate (NH_4PO_4)
4) Boric acid (H_3BO_3)
5) Calcium chloride dehydrate ($CaCl_2.2H_2O$)
6) Cobalt chloride ($CoCl_2.6H_2O$)
7) Copper(II) sulphate pentahydrate ($CuSO_4.5H_2O$)
8) Ethylenediaminetetraacetic acid disodium salt dihydrate ($Na_2MoO_4.2H_2O$)
9) Ferrous sulphate heptahydrate ($FeSO_4.7H_2O$)
10) Glycine
11) Hydrochloric acid (HCl)
12) Kelcogel
13) Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$)
14) Manganese sulphate monohydrate ($MnSO_4.H_2O$**)**
15) Myo-inositol
16) Nicotinic acid
17) Potassium dihydrogen phosphate (KH_2PO_4)

18) Potassium iodide (KI)
19) Potassium nitrate (KNO_3)
20) Pyridoxine HCl
21) Sodium chloride (NaCl)
22) Sodium hydroxide (NaOH)
23) Sodium molybdate ($Na_2MoO_4.2H_2O$)
24) Sucrose
25) Thiamine HCl
26) Zinc sulphate heptahydrate ($ZnSO_4.7H_2O$)

2.2. Reagents for enzyme analysis

1) ATP
2) Ammonium molypdate
3) Bovine serum albumin (BSA)
4) 1-chloro-2,4-dinitrobenzene (CDNB)
5) Copper (II) sulfate ($CuSO_4.5H_2O$)
6) Disodium phosphate
7) 1,4-dithiothreitol (DTT)
8) Ethylene diamine tetraacetic acid (EDTA)
9) Folin-Ciocalteau's phenol reagent
10) Glacial acetic acid (CH_3COOH)
11) GSH
12) Guaiacol
13) Hydrochloric acid (HCl)
14) Hydrogen peroxide (H_2O_2)
15) Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$)
16) Malachite green
17) p-nitrophenyl-2-D-glucopyranoside (PNPG)
18) Perchloric acid
19) Polyvinylpyrrolidone

20) Potassium dihydrogen phosphate (KH_2PO_4)
21) Potassium nitrate (KNO_3)
22) Potassium sodium (+)-Tartrate ($KNaC_4H_4O_6.H_2O$)
23) Sodium acetate
24) Sodium carbonate ($NaCO_3$)
25) Sodium carbonate anhydrous (Na_2CO_3)
26) Sodium hydroxide (NaOH)
27) Tris (hydroxyl methyl) aminomethane ($NH_2C(CH_2OH)_3$
28) Tween 20®

2.3. Reagents for proteome analysis

1) Acetone
2) Acetonitile
3) Acrylamide PAGE (CH_2:$CHCONH_2$)
4) Ammonium Persulfate
5) Bovine serum albumin (BSA)
6) Bromophenol blue sodium salt ($C_{27}H_{28}Br_2O_5S$)
7) Copper (II) sulfate ($CuSO_4.5H_2O$)
8) 1,4-dithiothreitol (DTT)
9) Ethylene diamine tetraacetic acid (EDTA)
10) Folin-Ciocalteau's phenol reagent
11) Glycerol ($CH_2OHCHOHCH_2OH$)
12) Glycine
13) Hydrochloric acid (HCl)
14) Iodoacetamide
15) 2-Mercaptoethanol ($HSCH_2CH_2OH$)
16) Methanol (CH_3OH)
17) N, N´ Methylenebisacrylamide ($(CH_2$:$CHCONH)_2CH_2$)

18) Potassium sodium (+)-Tartrate ($KNaC_4H_4O_6.H_2O$)
19) Silvernitrate
20) Sodium carbonate anhydrous (Na_2CO_3)
21) Sodium Dodecyl Sulfate ($C_{12}H_{25}NaO_4S$)
22) Sodium hydroxide (NaOH)
23) TEMED
24) N,N,N´, N´-tetramethylethylenediamine
25) Tris (hydroxyl methyl) aminomethane ($NH_2C(CH_2OH)_3$
26) Trypsin

3. Methods

3.1. Plant materials

The seeds of rice were dehusked and soaked in 70% ethanol for 30 seconds after that they were immersed in 5% Clorox® (5.25% sodium hypochlorite) with 0.1% Tween 20® for 8-12 h on an orbital shaker, and then repeatedly washed in 25% Clorox® with 0.1% Tween 20® for 25 min and rinsed three times by sterile distilled water. The sterilized seeds were germinated on the solidified MS medium (Murashige and Skooge, 1962) for a week under 25 ± 2^oC air-temperature, 60 ± 5% relative humidity (RH), and 60 ± 10 μmol $m^{-2}s^{-1}$ photon flux (PPF) with 16 h d^{-1} photoperiod. The coleoptile were cut into segments with 3-5 mm in length and placed on the embryogenic callus induction media for 4 weeks in the same above conditions but under dim light.

The friable embryogenic callus were selected and transferred to 250 ml Erlenmeyer flasks, each of which contained 50 ml liquid media and were subcultured weekly. The cultures were maintained at 25 ± 2 ^oC with continuous shaking at 110-120 revolutions per minute (rpm) on an orbital shaker. After the growth rate was constant in a linear phase, cell suspensions were treated with and without 513 mM sodium chloride (NaCl) for 0, 1, 3, 6, 12, 24, 48 and 72 h. The suspension cells were

harvested, washed with the distillated and deionized water and stored at -80 ^{o}C until further use.

The embryogenic callus induction media were prepared from MS medium stock supplemented with 50 mM 4-amino-3,5,6,trichloro-picolinic acid (picloram), 3% sucrose and Kelcogel 2.5 g/l. The liquid media were prepared from SH stock (Schenk and Hildebrandt, 1972) with 17 mM picloram and 3% sucrose. All media were sterilized by autoclave for15 min, at 15 psi, 121^{o}C.

3.2. Growth analysis

The cell growth was analyzed by collecting the cell suspension every 24 h for 2 weeks and centrifuged at 10,000 rpm. The cell fresh weight was measured and was dried at 110 ^{o}C for 24 h. The dry weight and fresh weight (Y-axis) was plotted against culture time (X-axis) in the growth curve to find out the linear phase of growth. All experiment did five replications.

3.3. Enzyme activity analysis

One hundred milligrams of suspension cell were collected and grounded with mortar and pestled by using liquid nitrogen until fine powder was produced. The powder was transferred to 1.5 ml micro-centrifuge tube added with 500 µl of 0.1 mM Tris-HCl, pH 7.0, 2 mM ethylenediaminetetraacetic acid (EDTA), 4 mM 1,4-dithiothreitol (DTT) and 60 mg/ml polyvinylpyrrolidone as an extraction buffer. The samples were sonicated in sonicator (Ultrasonic Cleaner, Sonorex RX-100) for 15 min, the supernatant was carefully collected by centrifugation at 12,000 rpm at 4 ^{o}C for 15 min (refrigerator micro-centrifuge, Jouan). The supernatant were stored at 4 ^{o}C until used as the crude extract.

1) Protein determination by Lowry assay

The protein content in crude extract was determined according to Lowry *et al.* (1951) by using the bovine serum albumin (BSA) as the protein standard for creating the protein standard curve. Ten microliters of crude extract was pipetted into 96 well micro titer plate (Nunc^{TM}, Denmark) and added with 200 µl of Lowry A reagent. The mixture was incubated at room temperature for 30 min and then added with 50 µl of the Lowry B reagent. Then the mixture was kept at room temperature for 30 min. After that the mixture was measured at 750 nanometer of the wavelength by spectro-microplate reader (SpectraMax M5 multi-detection). The protein concentration was calculated by using the standard curve which plotted between OD_{750} on the Y-axis and series of BSA concentrations (µg/ml) on the X-axis.

2) Glucosidase activity assay

The glucosidase activity in crude extract was determined by the modified method of Lymar *et al*. (1995). Five microliters of crude extract were pipetted into 96 well micro titer plate and added with 200 µl of 1 mM p-nitrophenyl-2-D-glucopyranoside (PNPG) in 100 mM sodium acetate pH 5.0. The mixture was incubated at 30 ^{o}C for 10 min and the reaction was stopped by 50 µl of the 5 mM sodium carbonate ($NaCO_3$). The mixture was measured at the wavelength 400 nanometer (Extinction coefficient at 400 nm = 18.3 $mM^{-1}cm^{-1}$) by spectro-microplate reader. One unit of glucosidase activity is defined as that amount of enzyme which will hydrolyze 1 µmol of PNPG per minute at 30 ^{o}C.

3) Peroxidase activity assay

The peroxidase activity in crude extract was determined by the modified method of Nakano and Asada (1981). Five microliters of crude extract was pipetted into 96 well micro titer plate and 200 µl of substrate solution (prepared from 0.174 mM H_2O_2, 2.358 mM guaiacol) in other buffer pH 5.0, 7.0 and 10.0 as acidic

peroxidase, neutral peroxidase and basidic peroxidase respective, was added. The mixture was measured immediately under the light absorbance at the wavelength 436 nanometer (Extinction coefficient at 436 nm = 25.5 $mM^{-1}cm^{-1}$) by spectro-microplate reader every 30 seconds for 3 min. One unit of peroxidase activity is defined as 1nmol of tetraguaiacol formation per minute per mg protein using 2.358 mM guaiacol and 0.174 mM H_2O_2 at 25 ^oC.

4) Adenosine triphosphatase (ATPase) activity assay

The adenosine triphosphatase (ATPase) activity assay at plasma membrane (P-type) and vacuolar (V-type) were determined by the modified method of Madhu *et al* (2001). Potassium dihydrogen phosphate (KH_2PO_4) was used as the released phosphate standard for creating a phosphate standard curve. Five microliters of the crude extract was pipetted into 96 well micro-titer plate and added with 50 µl of substrate solution (prepared from 100 mM Tris-HCl pH 6.5, 50 mM $MgSO_4$, 50 mM ammonium molypdate, 15 mM ATP, 1.15 perchloric acid, 1.5% Tween 20® and 1 M KNO_3 for P-type while without KNO_3 for V-type). The mixture was incubated at 37 ^oC for 30 min and added with 200 µl of the colour reagent (prepared from 8.5 mM ammonium molypdate, 1.62 mM malachite green, 0.2 M 60% perchloric acid and Tween 20®). The liberated inorganic phosphate was suddenly measured under the light absorbance at the wavelength 660 nanometer by spectro-microplate. One unit of ATPase activity will liberate one µmol of the inorganic phosphate from ATP per minute at 37 ^oC.

5) Statistical analysis

The results presented the mean values with standard deviations of replication number (n = 3). The significance of differences between rice cultivar (PK, IR29, HJ, KDML105, PT1), treatment condition (control and salt stress) and incubation time (0, 1, 3, 6, 12, 24, 48 and 72 h) were analyzed using one way

ANOVA at the level of significance of $P < 0.01$ and <0.05. For enzyme analysis was done on a Completely Randomized Design (CRD).

3.4. Proteomic analysis

1) Protein extraction and determination

Three hundred milligrams of the cells from the culture were collected by centrifugation. The cell pellets were washed with deionized distillated water 3 times and grounded with mortar and pestles by using liquid nitrogen until fine powder was produced. The powder was transferred to 1.5 ml microcentrifuge tube and 900 µl of 0.1% SDS was added into the tube and incubated on incubator shaker for 1 h. The supernatant as the protein extract was carefully collected by centrifuged at 12,000 rpm, 4 ^{o}C for 20 min and stored at -20 ^{o}C until used. The protein extract was precipitated overnight with cold acetone at -20 ^{o}C and the precipitate was collected by centrifuging at 12,000 rpm, 4 ^{o}C for 20 min for determination of the protein concentration according to Lowry *et al.* (1951).

2) Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE)

Proteins were size-fractionated on SDS-PAGE mini slab gel (90 mm x 80 mm x 0.75 mm) according to the manufacturer method (Bio-Rad, US). The separating gel at the bottom and the stacking gel on the top of SDS-polyacrylamide gels were prepared by the following method. The separating and stacking gel were prepared from 12.5% and 4% acrylamide, respectively. Five micrograms protein from the extract with 5 microliter of 5X SDS loading buffer (prepared from Tris-HCl pH 6.8, 20% glycerol, 5% SDS, 0.2M DTT, 0.02% bromophenol blue) were heated at 95 °C for 10 min and was loaded onto the stacking gel which conducted at 30 volts and 70 volts in separating gel. The molecular masses of protein bands were estimated by comparing to the low molecular mass markers (Amersham Biosciences, UK). The electrophoresis was conducted in running buffer (25 mM Tris-HCl pH 8.3, 192 mM

glycine and 0.1% SDS) until the dye front reached the bottom of the separating gel. At the end of the run, the gel was removed from the cassette before staining with silver nitrate according to Blum *et al*. (1987). The protein gel was fixed in the fixing solution (50% methanol, 12% acetic acid and 50 µl of 37% formaldehyde to 100 ml fixing solution) for 30 min. The gel was removed and placed in the washing solution (35% ethanol) twice for 5 min each and sensitized in 0.02% sodium thiosulfate for 2 min. After washing in water twice for 5 mins each, the gel was stained with 0.2% silver nitrate for 20 min and was shaken in the developing solution (6% Na_2CO_3 w/v, 0.04% $Na_2S_2O_3$ v/v, 37% formaldehyde) until developed protein bands were visualized and the developing reaction was terminated quickly in the stopping solution (1.46% w/v sodium EDTA) for 20 min. The gel was scanned by a scanner (GE Healthcare). Gel image were carried out using Quantity One (GE Healthcare) and the gel was kept in 0.1% acetic acid at 4 ºC.

3) Protein digestion and peptide analysis by Liquid Chromotrography-Mass Spectrometry (LC-MS)

A wide range of molecular masses were determined by the standard protein markers. The excised gel bands were ranged according to the molecular mass as the followings:

Group 1: Molecular mass range $x \geq 97$ kD
Group 2: Molecular mass range $97 > x \geq 66$ kD
Group 3: Molecular mass range $66 > x \geq 45$ kD
Group 4: Molecular mass range $45 > x \geq 30$ kD
Group 5: Molecular mass range $30 > x \geq 20.1$ kD
Group 6: Molecular mass range $x < 20.1$ kD

The gels were washed, reduced, alkylated and digested with trypsin (Promega, USA) by following Leammi (1970). The peptides eluted from electrophoretic gel were injected into Ultimate 3000 LC System (Dionex) coupled to Electrospray ionization (ESI)-Ion Trap MS (HCT ultra PTM Discovery System,

Bruker Daltonik). The sample was separated on a nanocolumn (Onyx monolithic HDC18, 0.2 mm i.d. × 150 mm) at flow rate of 1.6 µl/min. A Solvent gradient (Solvent A: H_2O, 0.1% Formic acid; Solvent B : 20% H_2O, 80% Acetronitrile, 0.1% Formic acid) was started with 10% - 70% B at 0-13 min, 90% B at 13-15 min and 10% B at 15-20 min. All obtained MS/MS ions were analyzed with DeCyderMS Differential Analysis software (DeCyderMS, GE Healthcare) and submitted to the protein search engine MASCOT (Matrix Science Ltd., London, UK) against NCBIs database. Individual protein data generated by MASCOT were first cut off by using fold filter of signal intensity (Both >2 fold and < 2 fold). The data set were estimated for the predictive power of a clustering algorithm by Figures of Merit: FOM (Yeung *et al.* 2001). The cluster number which fitted to the expression patterns were used as input for k-means clustering (KMC) implemented through the multi-experiment viewer gene expression analysis package. The KMC was conducted by using a Pearson Correlation metric. The hierarchical trees showed each protein which is represented by a single row of colored boxes, and each time point is represented by a single column. Induction (or repression) ranges from pale to saturated red (up-regulation) or green (down-regulation). For each cluster, the cluster number was given in the right bottom corner, the number of proteins presented in the left upper corner. The different expression profile of these clusters played an important function on the salt stress against mechanisms. PK variety was used as the salt tolerant model and IR29 was used as the salt sensitive model. The expression profile of each individual protein in the cluster is depicted by grey lines; the mean expression profile is marked in pink.

RESULTS AND DISCUSSION

Results

1. Growth analysis of suspension cells

Steriled seeds of 5 varieties were grown in solidified MS medium. After 5-7 days of germination, the scutellum was cut and placed on solidified MS mediums supplemented with 50 mM picloram (callus induction medium). The explants swelled and initiated into the callus in one week. Nonembryogenic callus and somatic embryogenic callus were formed in the induction medium after 4 weeks. The nonembryogenic callus was translucent white and turned brown later. In contrast, the somatic embryogenic callus composed of a cluster of friable cells which were yellowish and had the globular shape as shown in Fig. 6.

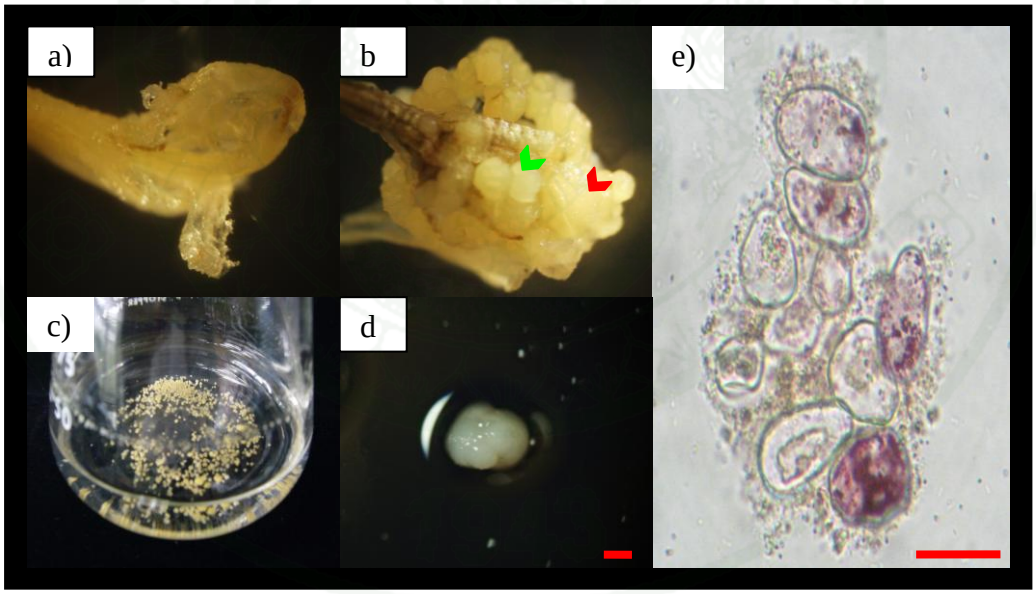

Figure 6 Characteristic of Pathumthani 1 callus when cultured on solidified induction medium for a month. The green arrow head pointed the embryogenic callus and the red arrow head pointed the nonembryogenic callus (a-b). The embryogenic callus were cultured in liquid medium and aggregated into the small cell clumps (c-d). The suspension cell under microscope (e). Bar: 200 µm

This friable embryogenic callus which obtained in the experiment was in good quality for further suspension culture. The suspension cells of the salt tolerant and salt sensitive rice had the similar profile of growth. The growth curve based on dry weight were observed, Three phases of growth were observed, exponential phase (days 0-5), linear phase (days 6-9), and stationary phase (after 10 days) (Fig. 7). In the exponential phase, the growth rate of the suspension cells was slow at the first 3 days of culture (lag phase) because of the low number of inoculum. The growth rate was exponentially increased 4-5 days after the beginning of the culture. The dry weight as an indicated parameter of growth increased about 2-fold over the initial. From the 6^{th} to the 9^{th} day, the dry weight increased linearly in the linear growth phase. The growth rate was constant after the cells were cultured approximately 10-14 days. The growth of suspension cell decreased and most of the cells became brown after two weeks. The characteristics of cells in suspension culture were creamy white or yellowish with aggregation of individual single cells or small clump of cell (Fig. 6). In this study, all varieties showed the similar pattern of the sigmoid growth curve both of fresh weight and dry weight. However, the phase separation of dry weight was more clearly seen than that of fresh weight.

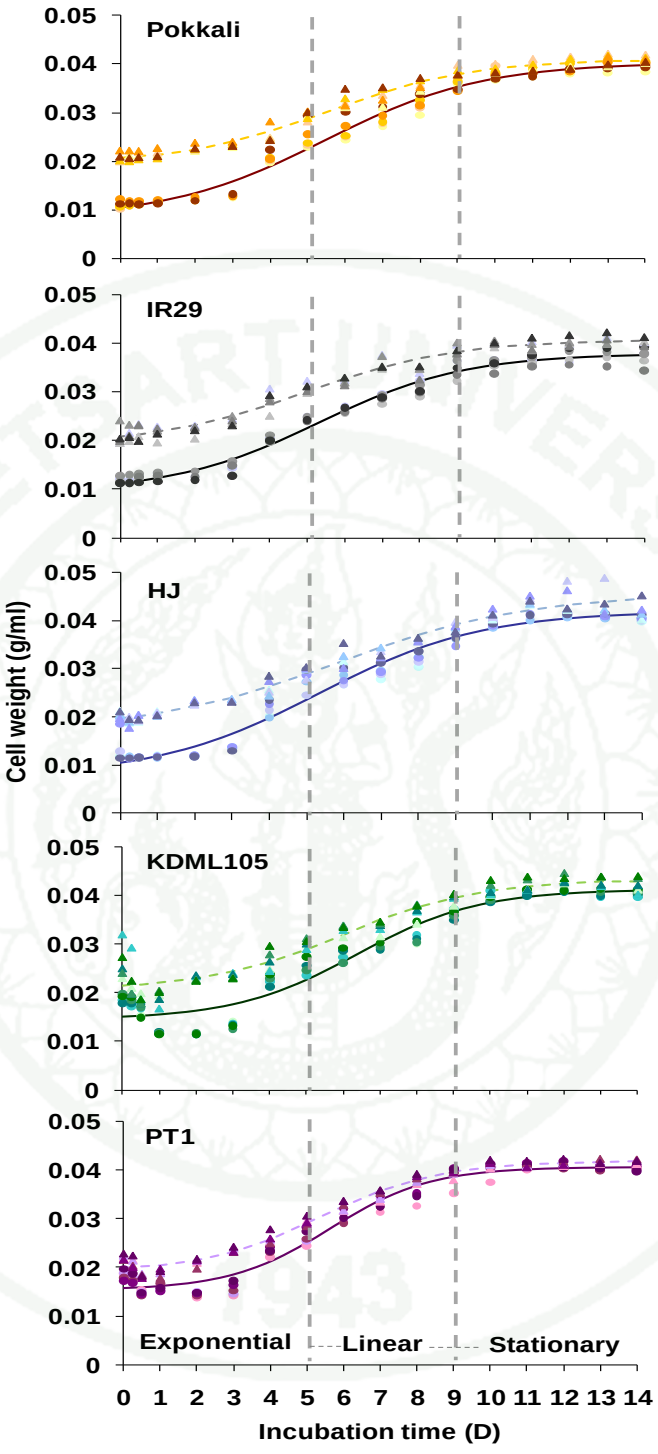

Figure 7 The growth curve of suspension culture for 2 weeks. The growth curves were plotted with the cell fresh weight (-▲-) and the cell dry weight (-●-) showed 3 phases as exponential phase (days 0-5), linear phase (days 6-9), and stationary phase (after 10 days).

2. Enzyme activity analysis

In comparison to control, salt stress induced a significant different expression of relative protein content. Furthermore, the incubation time and rice cultivars could induce the significant decrease of the relative protein content as shown in Fig. 8. The decreased of relative protein content of PT1 was statistically different from IR29, PK, HJ and KDML105, whereas the decrease of the relative protein content of IR29 was similar to PK and HJ, the decrease of relative protein content of KDML105 was only similar to HJ.

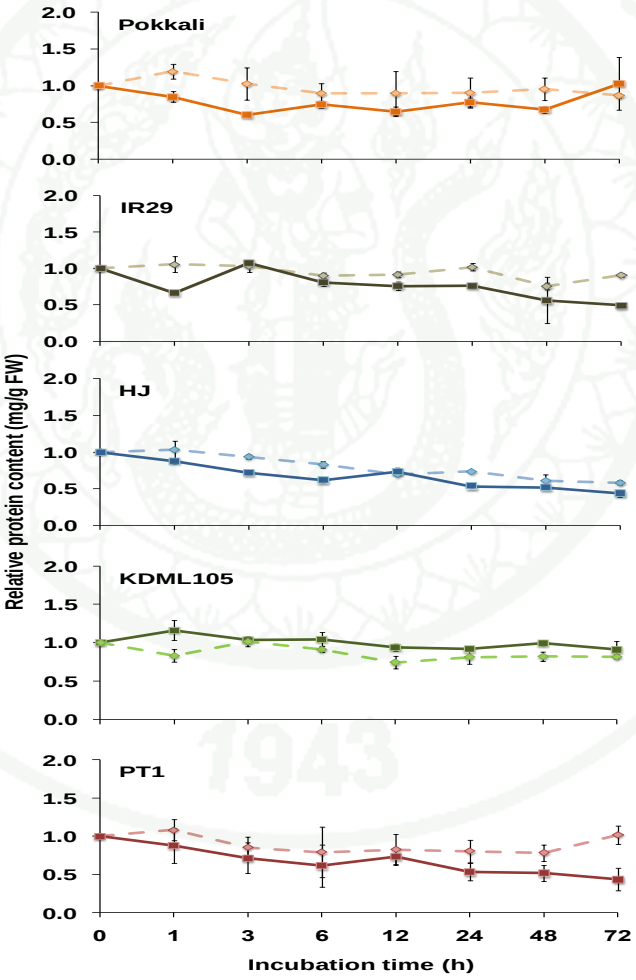

Figure 8 Relative protein content of cultured cells in NaCl stress (—●—) and control (- -●- -). The decreased of relative protein content at the significantly of control and NaCl stress ($p < 0.01$).

The relative activity of β-glucosidase was monitored at 0, 1, 3, 6, 12, 24, 48 and 72 h of the control and the salt stress treatments. In comparison to the control of cultured cells, salt stress induced a significant expression of β-glucosidase activity. The IR29 and PT1 cultivars significantly increased of β-glucosidase activity, in contrast, the activity significantly decreased in HJ, PK and KDML105. Nevertheless, the incubation time did not affect the activity of β-glucosidase in 5 varieties (Fig. 9).

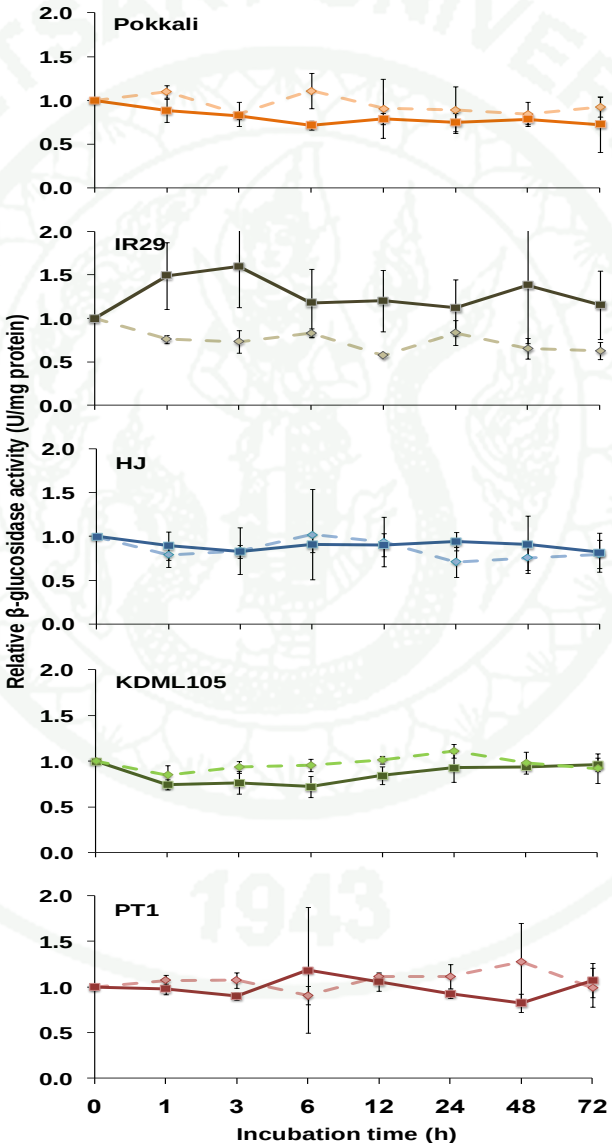

Figure 9 Relative β-glucosidase activity of cultured cells in NaCl stress (—●—) and control (-●-). The significant different expressed of control and NaCl stress ($p < 0.01$).

Three isoforms of peroxidase such as the acidic peroxidase, neutral peroxidase and basidic peroxidase showed in the different pH (Fig. 10). The 5 rice varieties, showed the significant different expression of peroxidase activity. The highest increasing activity of the acidic peroxidase and neutral peroxidase was found in salt-sensitive PT1, whereas the highest increasing activity of the basidic peroxidase was found in salt-tolerant PK. Furthermore, the increasing of incubation time showed the increasing of the peroxidase activity in every isoforms.

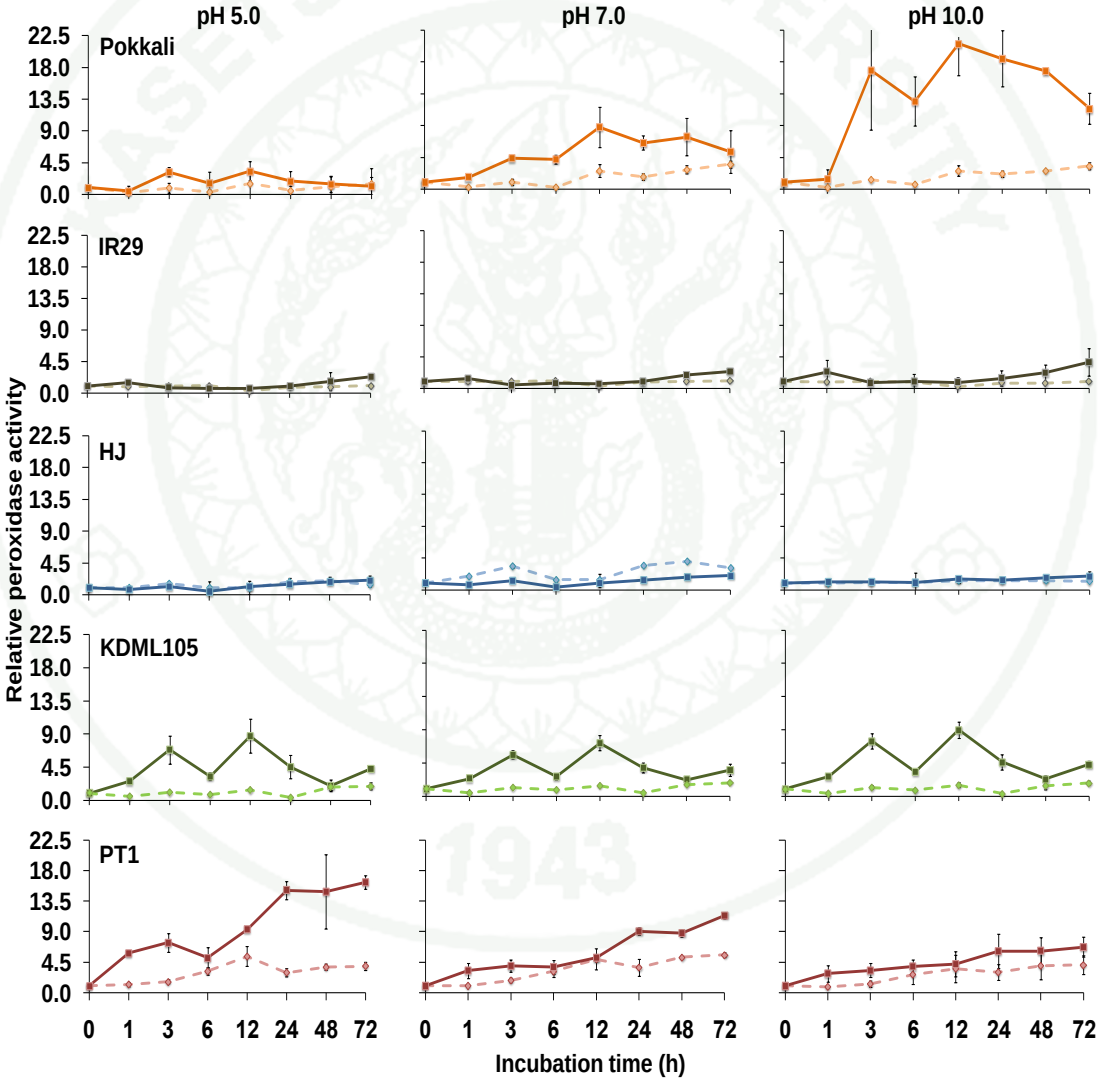

Figure 10 Relative peroxidase activity of cultured cells in NaCl stress (—●—) and control (-●-). Each isoform showed the increased of relative peroxidase activity at the significantly of control and NaCl stress ($p < 0.01$).

Intracellular ATPase activity of each rice suspension cells was shown in Fig. 11. Both of total ATPase and plasma membrane ATPase activity expressed differently depending on the varieties but the activity did not differ between the control and the salt stress treatment. In the salt-tolerant PK, total ATPase activity increased and reached the maximum activity at 1 h after treated with NaCl whereas the minimum activity was reached at 72 h after salt stress in the salt-sensitive IR29. The HJ, PT1 and PK had the similar pattern of total ATPase activity. For the plasma membrane ATPase activity, the maximum activity at 12 h after treated with NaCl was found in PT1 whereas the minimum activity was reached at 72 h after salt stress in R29. This expression of IR29 was found differently from other cultivars, contrasting to PT1 and PK which had the similar expression pattern.

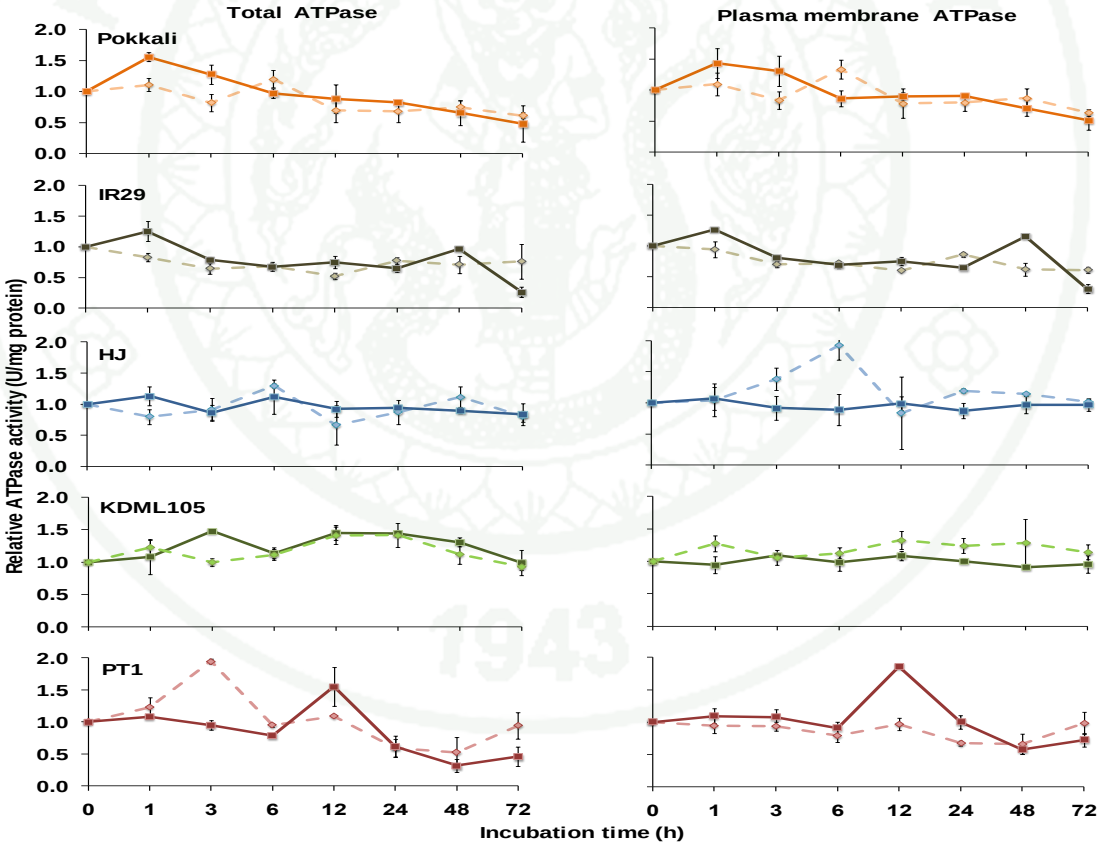

Figure 11 Relative ATPase activity of cultured cells in NaCl stress (—●—) and control (- -●- -). Both of total ATPase and plasma membrane ATPase activity expressed the different significantly depend on rice cultivar but the activity not differ between control and salt stress condition ($p < 0.01$).

3. Proteome analysis

From the LC-MS profile, many proteins were detected, matched and analyzed according to the DecyderMS software (Table 3). To achieve a comprehensive overview of the expression profile of the proteins that are co-expressed at different incubation times during salt stress, the fold filter was performed. After fold filter analyzed, totally 43 proteins were found. Thirteen clusters which fit to the expression patterns were determined by figure of merit (FOM, data not shown) and group arranged by k-means clustering (KMC) (Fig. 12-26).

In the salt-tolerant PK, the proteins in transcription and translation process, protein metabolism, sugar metabolism, respiration, and binding and transporting proteins were found under salt stress (Fig. 12). While the proteins involved in the transcription process (F-box domain containing protein), translation process (60s ribosomal protein I6L) and protein metabolism (leucine rich repeat containing protein kinase) were induced in the first time of salt incubation (PK-Ⅲ), NADH dehydrogenase subunit 5 which involved in respiration was down-regulated (PK-ⅩⅢ). The proteins such as myosin heavy chain, synaptobrevin-like protein, ankyrin-like protein, translation elongation factor EF-1 alpha, and aldolase C-1 involved in skeleton, transporting process, signal transduction, translation process and sugar metabolism respectively were depressed at 3 h after salt stress (PK-Ⅰ), the cell defense and response system proteins as glutathione s-transferase and peroxidase were induced at 3 and 6 h after salt stress (PK-Ⅶ and PK-Ⅳ). After salt stress 12 h, transcription factor protein was up-regulated whereas unclassified retrotransposon protein, translation initiation factor eIF-6, aspartyl protease, 60s ribosomal protein L24, elongation factor EF-2, SEC13-related protein and GTP-binding protein were down-regulated (PK-Ⅴ, PK-Ⅵ and PK-Ⅷ). Histidinol dehydrogenase and psi-h precursor (PK-Ⅺ) were not expressed under salt stress (Fig. 12).

In salt-sensitive IR29 (Fig. 13), all protein in sugar metabolism (aldolase C-1) and cell defense and response system proteins as peroxidase were induced in 1 h

after exposed to salt stress (IR-Ⅶ and IR-Ⅸ) including F-box domain containing protein, leucine rich repeat containing protein kinase, transcription factor, synaptobrevin-like protein, myosin heavy chain and histidinol dehydrogenase. However, the expression of these proteins was down-regulated in 1 h later. The expression of peroxidases, plasma membrane ATPase, protein ABIL-1, psi-h precursor, lysophospholipase homolog elongation EF-2, glycosyl transferase and 60s ribosomal protein were decreased in 6 h and increased after 12 h of salt stress induction. The up-regulation of proteins which involved in translation process (translation elongation factor EF-1 alpha), respiration (NADH dehydrogenase subunit 5), cell defense and response system proteins (glutathione s-transferase), transcription process (unclassified retrotransposon protein, homeodomain leucine zipper protein) and signal transduction (ankyrin like protein) were found in 6 and 12 h of salt stress (IR-Ⅺ, IR- Ⅻ and IR-ⅩⅢ) (Fig. 13).

Comparing to PK and IR29, proteins that involved in the translation process (translation initiation factor eIF-6 in HJ-Ⅵ), metal binding protein (opa-interacting protein in HJ-Ⅵ) and cell defense and response system (aquaporin in HJ-Ⅶ) in HJ was up-regulated rapidly in 1 h after salt stress whereas transcription factor (transcription process), aldolase C-1 (sugar metabolism) and protein ABIL 1 (skeleton) (HJ-Ⅸ and HJ-Ⅹ) were down-regulated. After salt stress 3 h, down-regulation of translation elongation factor, ankyrin like protein EF-1 alpha, NADH dehydrogenase subunit 5 and glycosyl transferase (HJ-Ⅲ and HJ-ⅩⅢ) were observed, while aquaporin (HJ-Ⅶ) and aldolase C-1 (HJ-Ⅸ) were up-regulated. The decreasing of protein ABIL-1 and increasing of translation elongation factor EF-1 alpha, ankyrin-like protein (HJ-Ⅲ), aldolase C-1, beta-glucosidase, histidinol dehydrogenase, glycosyl transferase and NADH dehydrogenase subunit 5 were found after salt stress induction 6 h. After 12 h of salt stress, the decreasing of translation elongation factor EF-1 alpha and elongation factor EF-2 were found. In contrast to the above proteins, the peroxidases were increased after 12 h of salt stress (Fig. 14).

In KDML105 (Fig.15), the up-regulated protein that involved in secondary metabolite metabolism, transcription process and translation process was found in 1 h after salt stress (cluster KD-XI) and reached the highest level after 3 h. In contrast to the above proteins, the proteins in cluster KD-II and KD-XIII which involved in transcription process, translation process, signal transduction and respiration were down-regulated. After 3 h of salt stress, the proteins (KD-X, KD-XI and KD-XII) that involved in skeleton and growth development, transcription process, translation process and cell defense and response system were up-regulated. After 48 h of salt stress, the expression of NADH dehydrogenase subunit 5 (KD-XIII) and skeleton and growth development proteins (KD-X) in KDML105 were decreased. The increasing of the expression in transporting proteins, cell and defense response system, sugar metabolism and protein metabolism as synaptobrevin-like protein, myosin heavy chain, pathogenesis related protein PR-10b, zeatin O-glucosyltranferase, glycosyl transferase ypfP and proline rich family protein (KD-IV and KD-VII) were found (Fig. 14).

In PT1 (Fig 15), the down-regulated proteins that involved in cell and defense response system (cluster PT-VIII and PT-XI), membrane protein (PT-XII) and skeleton and growth development protein (PT-XIII) were found at 1 h after salt stress whereas the salt response proteins were quickly up-regulated in PT-II, PT-V, PT-VI and PT-VII. After 3 h of salt stress, the up-regulated proteins were found in cell defense and response system, transporting protein and sugar metabolism (PT-V, PT-VIII and PT-X) (Fig. 15).

Table 3 Forty-three differntially expressed proteins in rice cell suspension under salt stress by analyzed with DeCyderMS Differential Analysis software.

Protein	Accession number	Peptide	Match/ Score	Mass (Da)
Skeleton and cell organization				
Myosin heavy chain	gi|31193918	R.LMINNR.I + Oxidation (M)	25/30	170023
Protein ABIL1	gi|255541748	K.AGPVS.-	1/27	34537
Zeatin O-glucosyltransferase	CA764857	R.TLTLGQGSR.S	1/12	25912
Sugar metabolism				
Aldolase C-1	gi|786178	K.VSPQLIAEYTVR.A	7/137	39141
Beta-glucosidase				
Histidinol	CA763212	-.SFFOA.-	1/10	26061
Glycosyl transferase ypfP	gi|27547464	K.GSASPWQAAAGACGCPSRCR.T	1/5	29011
Secondary metabolite metabolism				
Flavonol 4'-sulfotransferase	gi|27547220	-.KPCMXKR.X + Oxidation (M)	1/9	67582
NAD(P)H dependent 6'-deoxychalcone synthase	gi|27921023	K.MLYFLF.- + Oxidation (M)	1/4	79838
Transcription process				
Em binding protein-1a	CA757353	-.LETAH.-	1/8	22861
F2K11.18	gi|25803017	-.PSAQT.-	1/9	25432
F-box domain containing protein	gi|77556383	.GLGLCGCAINHGVISR.A	1/1	67301
HD-Zip protein	gi|25802926	R.YAFTKVVFI.-	1/3	78151
Homeodomain leucine zipper protein	gi|25799785	-.LRPNII.-	1/9	22392
Lysophospholipase homolog	CA760360	-.FCMAQLSQ.- + Oxidation (M)	1/7	26565
Opa-interacting protein	gi|25806125	R.KEEASDY.-	1/9	25333
Psi-h precursor	BE040299	-.LSSNNGASS.-	1/5	22334
Transcription factor	gi|25803301	K.ALGSH.-	1/10	62034
Unclassified retrotransposon protein	gi|242117496	K.WSLARNFK.A	4/42	145798

Table 3 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)
Translation process				
Elongation factor ef-2	BE040066	R.HMRAG.- + Oxidation (M)	1/2	35389
60s ribosomal protein l6	gi|8334650	R.RPGSH.-	1/5	21429
60S ribosomal protein L24	CA756401	R.LNVDIFD.-	1/4	23148
Translation elongation factor EF-1 alpha	CA753016	K.RGLEX.-	1/6	25917
Translation initiation factor eIF-6	CA763564	R.SPPCLGTGH.-	1/3	26761
Protein metabolism				
Aspartyl protease	gi|25797766	K.KKNRMK.Q	1/17	22163
Leucine rich repeat containing protein kinase	gi|48716959	K.TDYESNLTVQ GTQQT.-	1/6	114163
Proline-rich family protein	gi|18398103	R.SKHGMFGGK. R + Oxidation (M)	1/18	18383
Cell defense and rescue system				
Acidic peroxidase				
Aquaporin	gi|8334728	-.IKGCIM.-	1/1	100816
Basidic peroxidase				
Glutathione s-transferase	gi|25798958	K.KKSTAGGR.S	2/12	22473
Neutral peroxidase				
Pathogenesis-related protein PR-10b	CA759435	R.RAXRLGVGGA GVEGLSDAPAM PK.V	1/11	24168
Plasma membrane ATPase				
Total ATPase				
Transporting				
GTP-binding protein	CA757475	K.MQIQLH.- + Oxidation (M)	1/17	23646
p125 protein	gi|25799240	R.CPTGSPR.R	1/7	23044
SEC13-related protein	CA754964	-.YRFTIS.-	1/16	23420
Synaptobrevin-like protein	BE040833	R.ADAGGGGGGR .R	1/27	30595

Table 3 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)
Energy metabolism (respiration)				
NADH dehydrogenase subunit 5	CA756211	R.ELNGN.-	1/3	21618
Signal transduction				
Ankyrin-like protein	BU099192	R.ELGPGL.-	1/16	12208
Unknown				
agCP5654	gi|25803664	R.LFENKY.-	1/12	26289
OSJNBa0014K14.4	gi|70663918	M.APPLLPRGAAL	1/1	68298

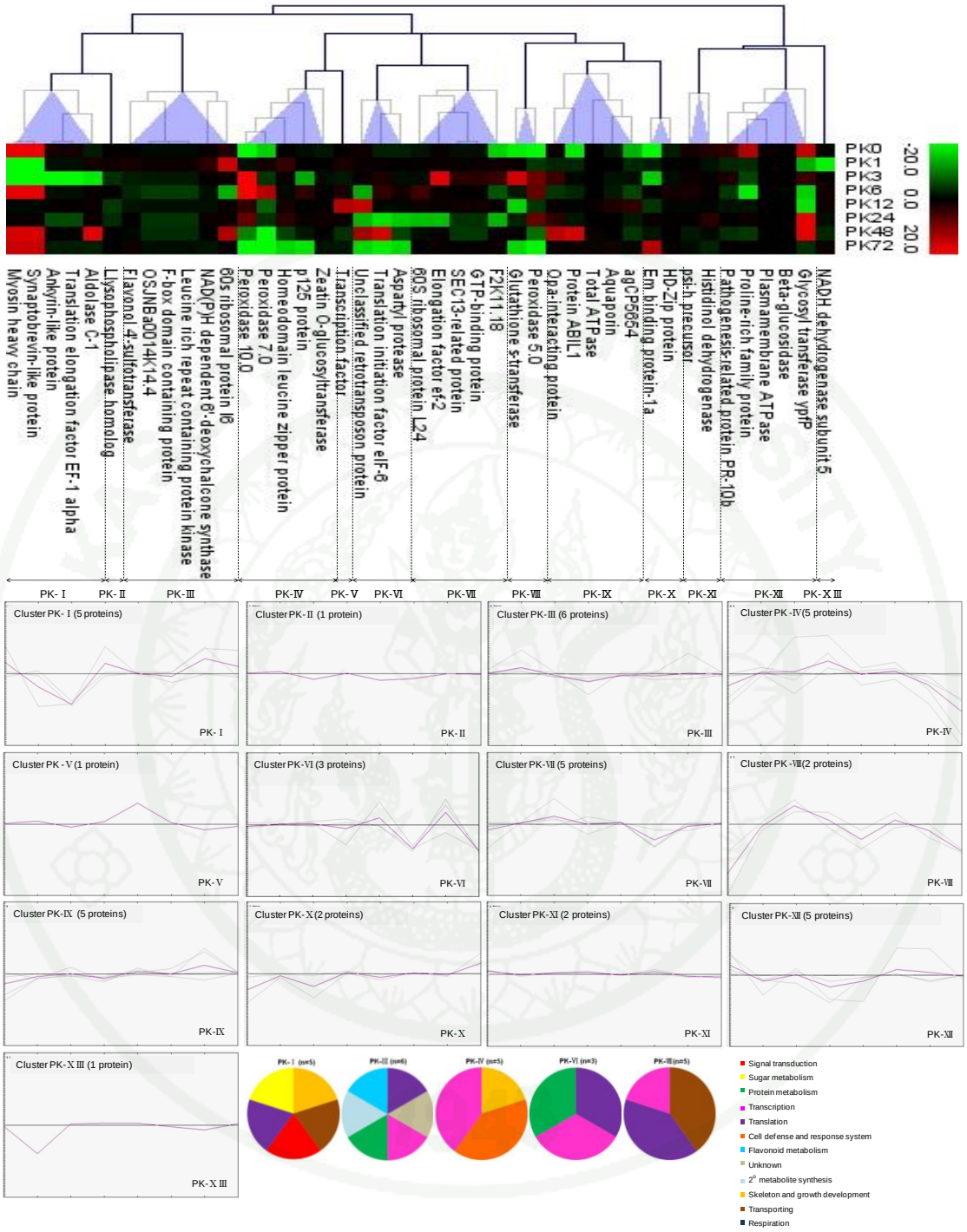

Figure 12 Clustering analysis of protein expression profiles during PK suspension cell were exposed to salt stress. The salt stress up-regulated proteins in cluster PK-Ⅰ, PK-Ⅲ, PK-Ⅴ, PK-Ⅵ, PK-Ⅶ, PK-Ⅷ and PK-Ⅸ whereas the down-regulation was found in PK-Ⅰ, PK-Ⅱ, PK-Ⅳ, PK-Ⅵ, PK-Ⅶ, PK-Ⅷ, PK-Ⅹ, PK-Ⅻ and PK-ⅩⅢ.

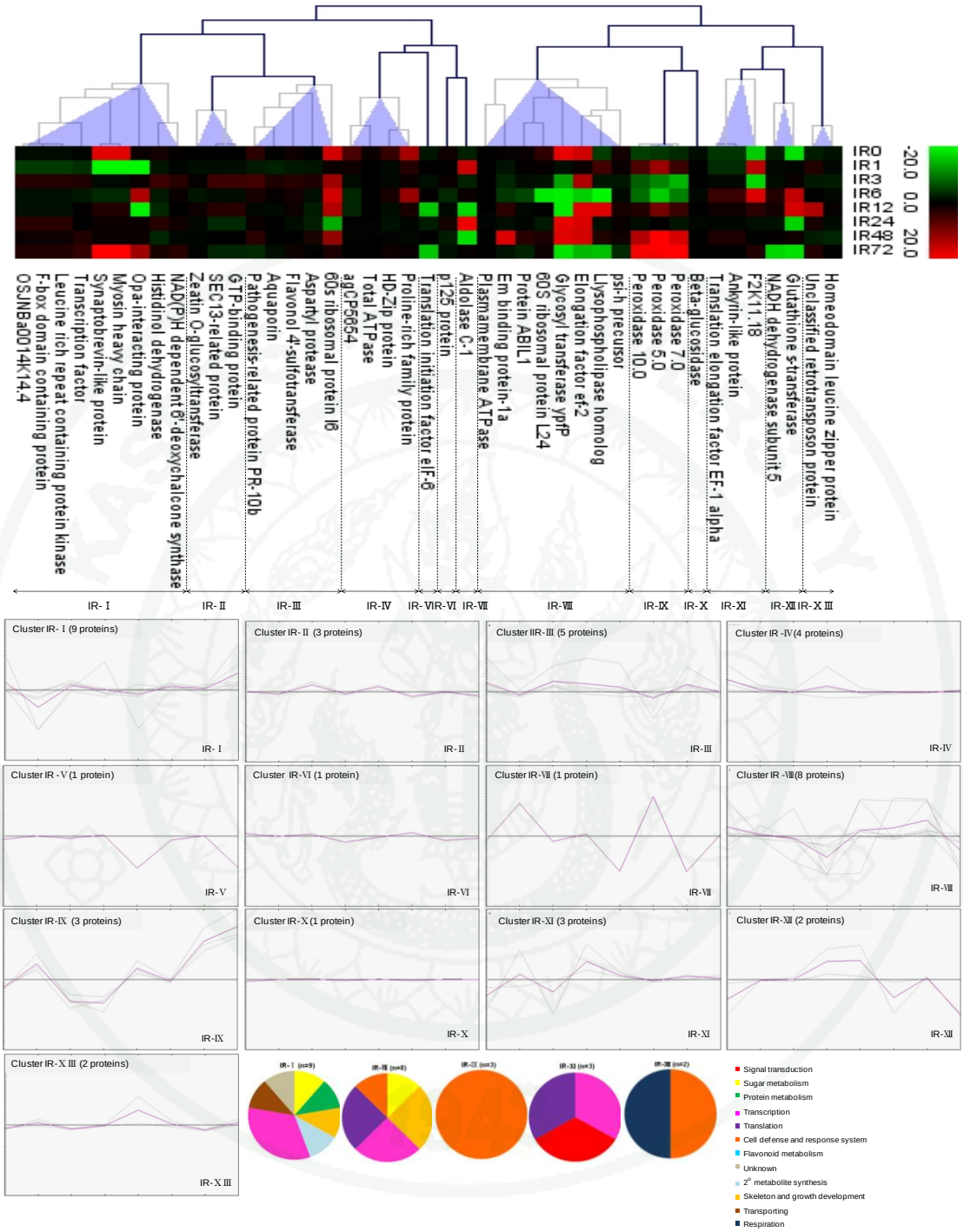

Figure 13 Clustering analysis of protein expression profiles during IR29 suspension cell were exposed to salt stress. The salt stress up-regulated proteins in IR-Ⅰ, IR-Ⅱ, IR-Ⅲ, IR-Ⅶ, IR-Ⅷ, IR-Ⅸ, IR-Ⅺ, IR-Ⅻ and IR-ⅩⅢ whereas the down-regulation was found in IR-Ⅰ, IR-Ⅴ, IR-Ⅶ, IR-Ⅷ, IR-Ⅸ, IR-Ⅺ and IR-Ⅻ.

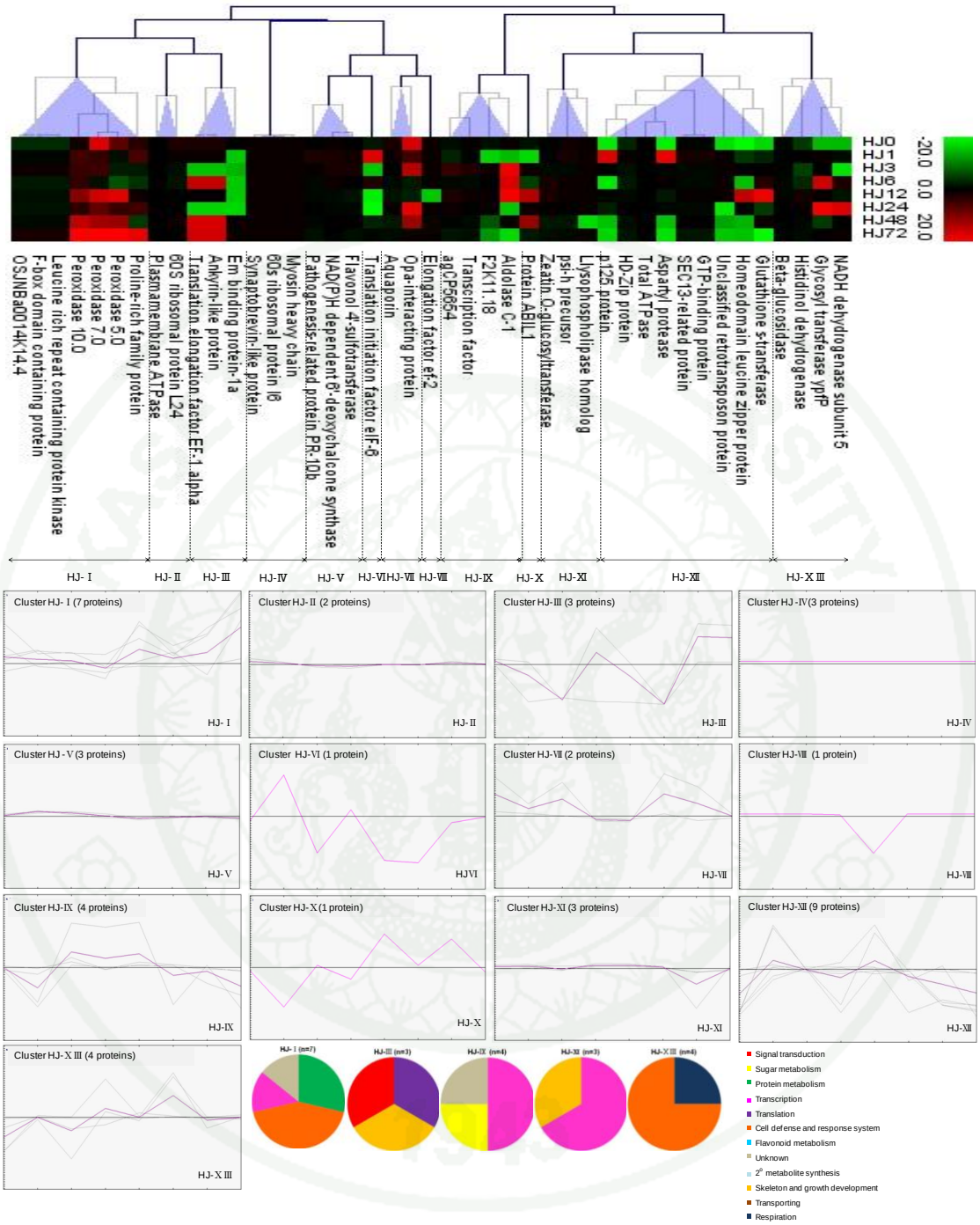

Figure 14 Clustering analysis of protein expression profiles during HJ suspension cell were exposed to salt stress. The salt stress up-regulated proteins in HJ-Ⅰ, HJ-Ⅲ, HJ-Ⅵ, HJ-Ⅶ, HJ-Ⅳ, HJ-Ⅹ, HJ-Ⅻ and HJ-ⅩⅢ whereas the down-regulation was found in HJ-Ⅲ, HJ-Ⅵ, HJ-Ⅷ, HJ-Ⅸ, HJ-Ⅹ, HJ-Ⅺ, HJ-Ⅻ and HJ-ⅩⅢ.

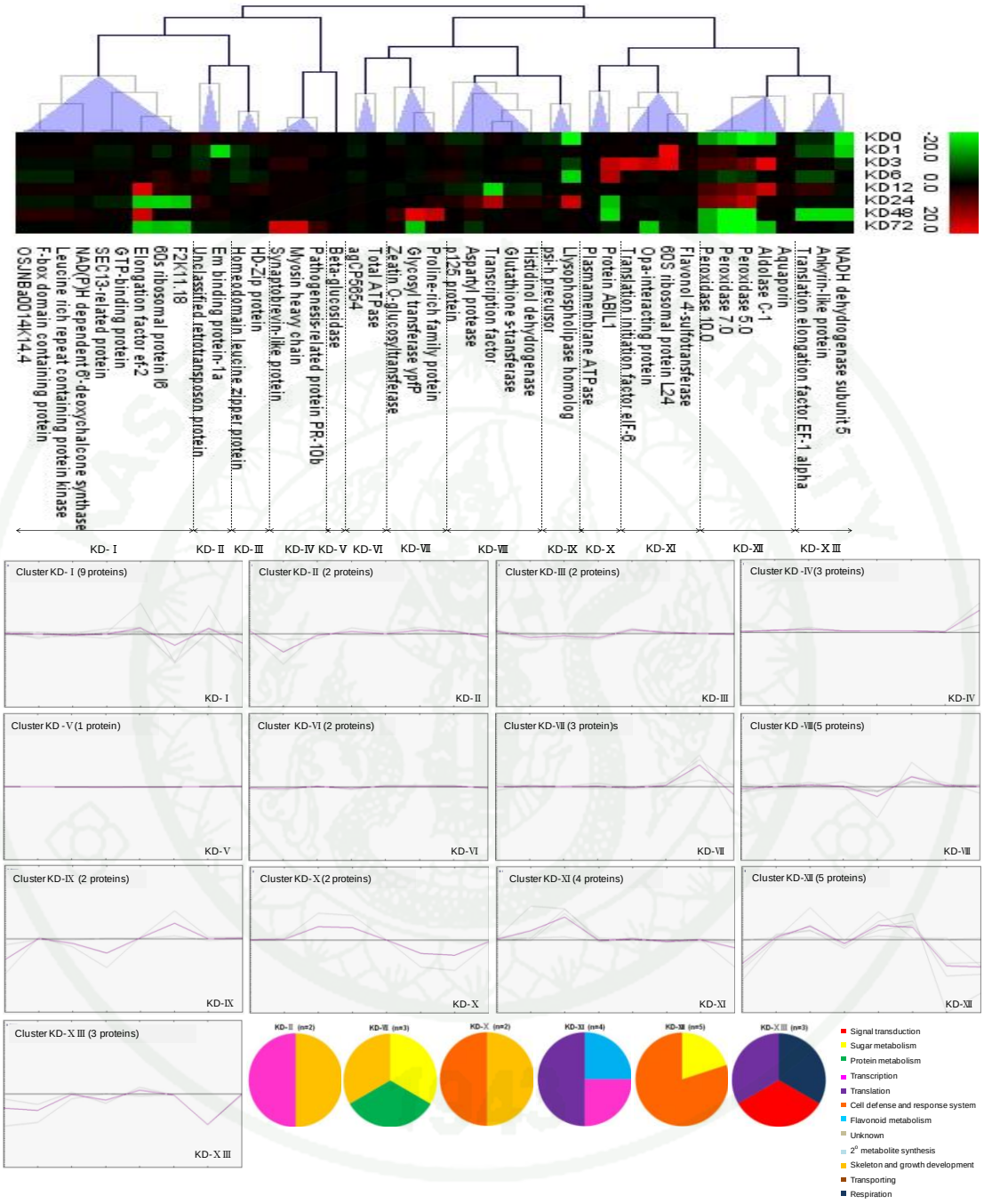

Figure 15 Clustering analysis of protein expression profiles during KD suspension cell were exposed to salt stress. The salt stress up-regulated proteins in KD-Ⅲ, KD-Ⅶ, KD-Ⅷ, KD-Ⅸ, KD-Ⅹ, KD-Ⅺ and KD-Ⅻ whereas the down-regulation was found in KD-Ⅰ, KD-Ⅱ, KD-Ⅷ, KD-Ⅸ, KD-Ⅹ, KD-Ⅻ and KD-ⅩⅢ.

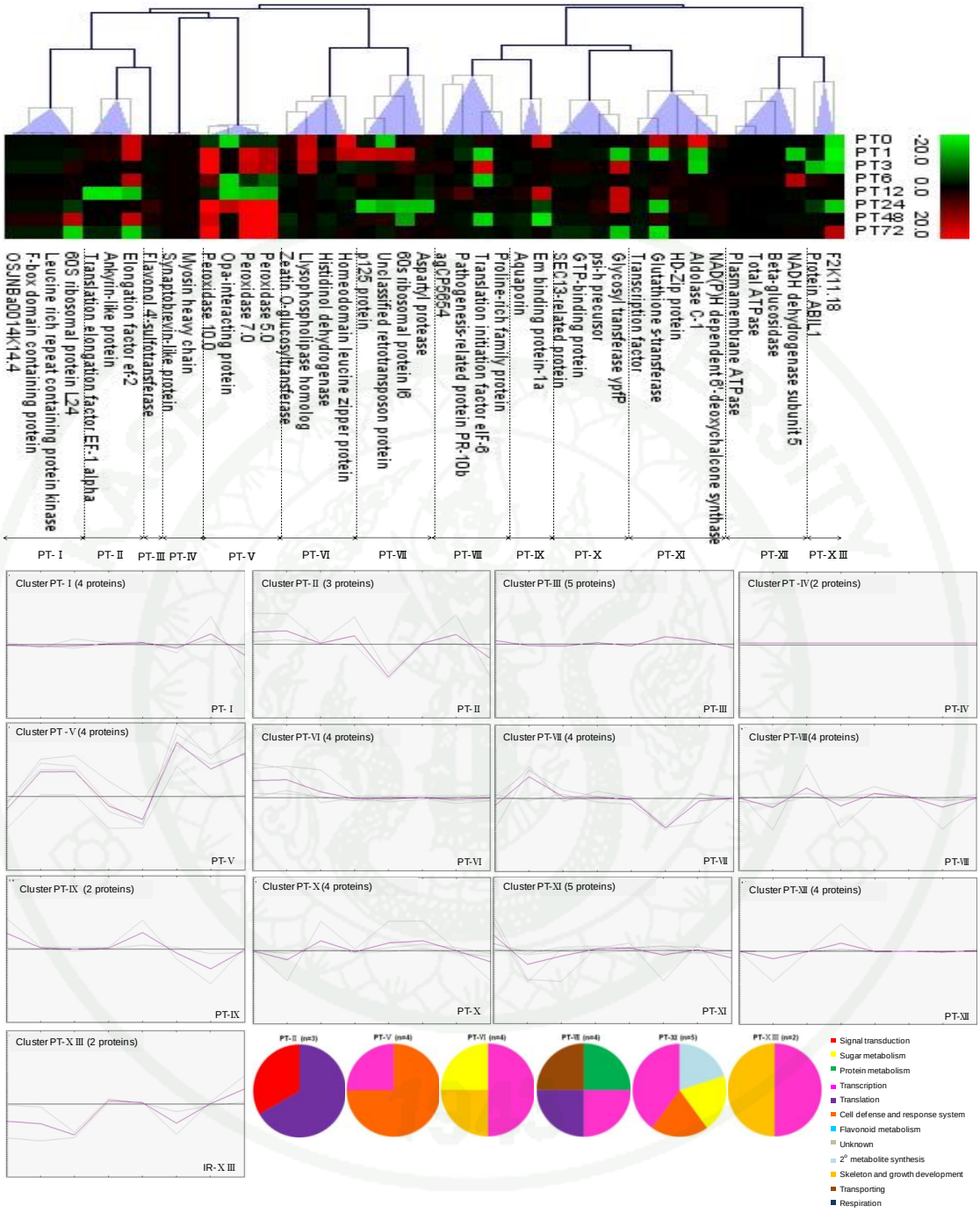

Figure 16 Clustering analysis of protein expression profiles during PT suspension cell were exposed to salt stress. The salt stress up-regulated proteins in PT-Ⅰ, PT-Ⅱ, PT-Ⅲ, PT-Ⅴ, PT-Ⅵ, PT-Ⅶ, PT-Ⅷ, PT-Ⅸ, PT-Ⅹ, PT-Ⅻ and PT-ⅩⅢ whereas the down-regulation was found in PT-Ⅰ, PT-Ⅱ, PT-Ⅴ, PT-Ⅶ, PT-Ⅷ, PT-Ⅸ, PT-Ⅹ, PT-Ⅺ, PT-Ⅻ and PT-ⅩⅢ.

Discussion

1. Growth analysis of suspension cells

Recently, plant cell suspension culture systems have been used for physiological and biochemical study. These culture systems have several advantages, including the multiplication of a number of plant cells and the reduction of cultivation time. It can be used to study the direct response of suspension cell to salinity without interferences of intact plant expressions. In the whole plant level, the different traits involved a complex phenomenon of responses which vary according to the developmental stage of rice plant (Akbar *et al.*, 1986; Vajrabhaya *et al.*, 1989). In cell culture, the embryogenic cells tolerated to the high level of salt than the other cell types (Binh *et al.*, 1992). Therefore, the homogeneous embryogenic rice cell suspension culture system was used in this study to establish a small model of growth and salt responding enzymes.

In this study, the solidified MS medium induced the large cluster of compact callus in one month and subsequently; the liquid SH medium induced the small cluster of cells and gave the embryoid-like structure. Besides the medium, the culture on an orbital shaker caused the loss of cell polarity and integrity. This resulted in differentiation of the compact callus to embryoid-like structure of the five rice varieties in two weeks.

In this study, adding of 50 mM picloram into MS medium increased growth of the somatic embryogenic callus of five rice varieties in one month. Picloram plays an important role on somatic embryogenic induction in the several plant species such as banana, sugar cane, casava and carnation (Sidha *et al.*, 2006; Garcia *et al.*, 2007; Bull *et al.*, 2009; Karami *et al.*, 2007). Similar to Sidha *et al.* (2006) and Stefanello *et al.* (2005) who found that 50 mM picloram effected the somatic embryogenic cell induction and gave the highest rate of proliferation and the high quality of somatic embryogenic cell compared with other auxins. The characteristic of sugarcane embryogenic cells in the medium containing picloram were originated nodular,

yellow, compact cells, dense cytoplasm and conspicuous nuclei, which is similar to banana somatic embryogenic cells (Garcia *et al.*, 2007 and Sidha *et al.*, 2006). For the reasons, picloram as an auxin, at the effective dose can stimulate RNA, DNA and protein synthesis leading to uncontrolled and disorganized cell division and growth (Tu *et al.*, 2001). Furthermore, picloram can serve the embryogenic callus more than 2,4-D that often used to the embryogenic plant cells induction and had the high regeneration potential in the suitable solidified media (Gonzalez *et al.*, 1998). However, the characteristic of embryogenic cells were not different in five varieties of rice, but the time for the differentiation was different. The salt-tolerant rice PK, KDML105 and HJ were initiated the embryogenic cells about one week faster than the salt-sensitive rice IR29 and PT1. This suggested that the salt-tolerant rice responded to picloram better than the salt-sensitive rice.

The growth curve of five varieties of rice was plotted between cell fresh and dry weight value against culture time (Fig. 7). All 5 varieties have the similar pattern of the sigmoidal growth curve. However, the growth curve from the dry weight gave a better pattern than that from the fresh weight. Similar to the study of Mustafa *et al.*(2011) who found that the dry weight gave the high accuracy of growth kinetic curve comparing fresh weight.

2. Enzyme activity analysis

Among the 5 varieties, significantly different expression of relative protein content was found in the control and the salt stress treatments. The relative protein content in these varieties was reduced after exposing to the salt stress. This indicated that the cellular system was damaged by salt stress. It has been found that salt stresses lead to the cabonylation of proteins in plant tissues such as pea (Romero-Puertas *et al.*, 2002), dry wheat (Bartoli *et al.*, 2004), *Arabidopsis* seeds (Job *et al.*, 2005) and rice leaf (Kristensen *et al.*, 2004). Under salt stress, there was an oxidation of amino acid side chains (protein carbonylation) e.g. proline and arginine to γ-glutamyl semialdehyde, lysine to amino adipic semialdehyde and threonine to aminoketobutyrate (Shringarpure and Davies, 2002). However, the reduction of the

relative protein content cannot be used as an indicator of the salt tolerant or salt sensitive rice because their content did not differ among the 5 varieties.

The activity of β-glucosidase, peroxidase and ATPase which involved in sugar metabolism, oxidation stress and ion homeostasis were studied. Under salt stress, the activity of β-glucosidase increased in salt-sensitive IR29 and PT1 and reduced in salt-tolerant PK, KDML 105 and HJ. These results indicated that β-glucosidase plays a role on the balance of osmotic potential in salt-sensitive IR29 (Khan *et al.*, 2010). The function of this enzyme is the hydrolysis of the terminal non-reducing beta-D-glucose residue to beta-D-glucose. Similar to the report of Siringam *et al.* (2011), who found that beta-D-glucose was increased under salt stress in the salt-sensitive PT1.

An immediately up-regulation of three isoform of peroxidase within an hour after treated with salt similar to the previously report in the cucumber seedling root (Du *et al.*, 2010), rice root (Cheng *et al.*, 2009), wheat (Meneguzzo *et al.*, 1999), sugar beet (Bor *et al.*, 2003) and sesame (Koca *et al.*, 2007). The up-regulation of antioxidative enzymes like peroxidase achieves a balance between the rate of formation and removal of ROS, and sustains H_2O_2 at the required levels for cell signaling (Turkan and Demiral, 2008). High level of H_2O_2 indicates the organelle injury from the salt stress and determines by the activity of peroxidase which catalysts H_2O_2 to H_20.

Nevertheless, the expression of ATPase activity in both salt-sensitive and salt-tolerant varieties had no effect under salt stress induction, the ATPase activity of salt-tolerant PK expressed earlier than in the ATPase activity of salt-sensitive IR29. In contrast, Serrano *et al.* (1999) found that the salt stress increased ATPase activity which resulted in the ion homeostasis in the cell. In the high-complexity, salt stress responsive mechanisms are referred to the ion homeostasis. During salt stress, in plant cells, there is a decrease in potassium (K^+) uptake and an increase in Na^+ influx. As Na^+ is toxic to some metabolic reactions, such as the Hal2p phosphatase in sulfate metabolism, and K^+ are the major solute contributing to osmotic pressure and ionic

strength, salt stressed plant cells must regulate cation transporters at the tonoplast and plasma membranes to maintain ion homeostasis (Serrano *et al*., 1989). Sodium toxicity is caused mainly by the similarity of the Na^+ and K^+ ions to plant transporters and enzymes. Plant cells typically maintain a high K^+/Na^+ ratio in their cytosol with relatively high K^+, in the order of 100-200 mM, and low Na^+, of about 1-10 mM (Blumwald *et al.*, 2000; Higinbotham, 1973). Thus, the efficient exclusion of Na^+ excess from the cytoplasm and vacuolar Na^+ accumulation are the main mechanisms for the adaptation of plants to salt stress. This is typically carried out by transmembrane transport proteins that exclude Na^+ from the cytosol in exchange for H^+, a secondary transport process which is energy-dependent and driven by the proton-motive force generated by the plasma membrane H^+-ATPase (Serrano, 1989), and by the vacuolar membrane H^+-ATPase and H^+-pyrophosphatase (Rea and Sanders, 1987; Rea and Poole, 1993).

3. Salt stress response proteins using proteomic analysis

Totally, 43 differentially expressed proteins were identified by LC-MS/MS and analyzed with DecyderMS software. Only 41 proteins showed high homology with known proteins existed in the NCBI database and the remaining 2 proteins are unknown (Table 3). The different 13 expression clusters based on their expression profiles were grouped by KMC method (Fig. 12-16). The proteins showed various functional groups after stimulation of rice suspension cells with high concentration of NaCl (Table 3). These proteins involved in protein metabolism, sugar metabolism, energy metabolism, transcription process, translation process, cell defense and response system, signaling transduction, metal binding proteins, transporting protein and skeleton and cell organization. Following KMC, we identified overrepresented GO terms within each cluster, which allowed construction of temporal profiles of overall cellular functions in response to salt stress.

The transcription proteins such as Em binding protein 1a, F-box domain containing protein, F2K11.18, HD-zip protein, homeodomain leucine zipper protein, lysophospholipase homolog, Opa-interacting protein, psi-h precursor, transcription

factor and unclassified retrotransposon protein were up-regulated under salt stress in both salt-tolerant and salt-sensitive varieties. These proteins are similar to the translation proteins such as elongation factor ef-2, 60S ribosomal protein l6, 60S ribosomal protein L24, translation elongation factor EF-1 alpha and translation initiation factor eIF-6 (Fig. 12). In the control treatment, these proteins did not express. Similar to Kawasaki *et al.* (2001), the result confirms that NaCl treatment affected the protein synthesis in two important steps via transcription and translation process.

Other up-regulated proteins which involve in protein metabolism, sugar metabolism, binding and transporting protein, cell defense and response system, signaling transduction, and skeleton and cell organization were related to the expression of transcription and translation proteins.

The up-regulation of transcription proteins were found immediately in 5 varieties of rice at 1 h after salt stress. In PK, the translation proteins increased after the increasing of transcription proteins while in IR29, HJ, KDML105 and PT1, the translation and transcription proteins increased in the same time. Similar to Kawasaki *et al.* (2001), this result indicated that the salt responded protein expression of the salt-tolerant PK was different from the salt-sensitive IR29, HJ KDML105 and PT1.

Under salt stress, there was the decreasing of translation elongation factor EF-1 alpha and elongation factor ef-2 in salt-tolerant PK and HJ and salt-sensitive IR29, KDML105 and PT1. Elongation factor may occur for synthesis of stress protein required for cell survival. The decreased level of the translation elongation factor EF-1 alpha and elongation factor ef-2 mRNA reduced the encoding for stress proteins synthesis (Hericourt and Jupin, 1999; Pandhal *et al.*, 2009). However, in PK and HJ the translation elongation factor EF-1 alpha and elongation factor ef-2 decreased although PK and HJ are salt tolerant cultivars. This indicates that other proteins may involve in the expression of stress protein.

Furthermore, salt stress leads to an increasing and decreasing of genes which encode ribosomal proteins synthesis. In *Synechocystis* (Kanesaki *et al.*, 2002), the ribosomal protein was increased while in *Thellungiella* and *Arabidopsis* the decreasing of ribosomal proteins (60S ribosomal protein L5, 60S ribosomal protein L5 L13A, 50S ribosomal protein L29 and 40S ribosomal protein S5) was found (Pang *et al.*, 2010). The changes in transcription and translation protein observed in this study suggest that the activity of protein synthesis may be particular importance in rice against salt treatment.

The up-regulation of ankyrin-like protein was found in PK, IR29, HJ, and PT1 after salt stress 12 h. The ankyrin protein is a cytoskeletal protein that located on the membrane of the endoplasmic reticulum in *Arabidopsis* cells and its function is not clear (Wei and Li, 2009). More recently, Lu *et al*. (2003) found that the function of this protein is a regulator and an effecter of salicylic acid signaling in the *Arabidopsis* defense response. The results indicate that salicylic acid signaling pathway enhanced under salt stress in PK, IR29, HJ and PT1 except KDML105 suspension cell.

Under salt stress, the NADH dehydrogenase subunit 5 was decreased in PK, HJ and KDML105 this indicates that there is a loss of electron in the respiratory chain which leads to oxidative stress (Pang *et al.*, 2010). Similar to Sobhanian *et al.* (2010) who reported that the NADH dehydrogenase was down-regulated under salt stress and led to a decrease in ATP pool of soybean seedling cells, as well as the study which showed the decreased expression of NADH dehydrogenase in *Synechocystis* under high salt (Pandhal *et al.*, 2009).

The oxidative stress which occurred from the down-regulation of NADH dehydrogenase subunit 5, caused the highly expression of peroxidase and glutathione s-transferase in the salt-tolerant PK, HJ and KDML105 to scavenging the reactive oxygen species under salt stress (Fig.12-16). Meanwhile, in the salt-sensitive IR29 the peroxidase and glutathione s-transferase were highly expressed. The increasing of peroxidase and glutathione s-transferase in the salt-sensitive IR29 did not involve with the decreasing of NADH dehydrogenase subunit 5 under salt stress. In

generally, ROS is generated from the un-pair electron in the biochemical reaction in the several organelles such as chloplast, peroxisome and mitochondria (Asada, 1999, Foyer and Noctor, 2005 and Turkan and Demiral, 2009). Therefore, the high activity of peroxidase and glutathione s-transferase in the salt-sensitive IR29 without the decreasing of NADH dehydrogenase subunit 5 might be the ROS was generated from the other organelle that does not mitochondria.

During salt stress, the salt-sensitive IR29 and PT1 showed the suddenly response to the ROS scavenging in the seedling of rice (Dionisio-Sese and Tobita, 1998; Vaidyanathan *et al.*, 2003; Cheng *et al.*, 2009 and Yan *et al.*, 2005), *Gossypium hirsutum* (Light *et al.*, 2005), sorghum (Jogeswar *et al.*, 2006), smooth cordgrass (Baisakh *et al.*, 2008), wheat (Wang *et al.*, 2003), cucumber (Du *et al.*, 2010), *Thellungiella* and *Arabidopsis* (Pang *et al.*, 2010). The functions of glutathione-s-transferase is described as a large and diverse group of enzymes, which catalyses the conjugation of electrophilic xenobiotic substrates with the tripeptide glutathione for exhibited efficient H_2O_2 scavenging mechanisms with significantly higher activities (Jogeswar *et al.*, 2006).

Moreover, five rice varieties expressed the up-regulation of salt responding proteins such as ATPase and aquaporin. These proteins are located on plasma membrane and functioned in water and proton transporting (Cheng *et al.*, 2009; Komatsu *et al.*, 2007; Baisakh *et al.*, 2008 and Wang *et al.*, 2003). Their activities expressed at different times from peroxidase and glutathione-s-transferase. This indicates that the ATPase and aquaporin are not involved in the same expression mechanism of peroxidase and glutathione-s-transferase in the salt responses.

It has become increasingly evident that plants response to the external environment and adapt rapidly to maximize survival opportunity and minimize risks. In this study, the increase in histidinol dehydrogenase, glycosyltransferase ypfP and aldolase C-1 in cellular metabolism were observed in the salt-tolerant PK, HJ and KDML105 and the salt-sensitive IR29, PT1 under salt stress. The Glycosyltransferases are able to recognize hormones, secondary metabolites, biotic

and abiotic chemicals and toxics in the environment (Choudhary *et al.,* 2009). The aldolase C-1 is an indicated enzyme in several stresses and plays an important role in the glycolytic pathway (Abbasi and Komatsu, 2004). Thus, their differential expressions suggest their potential roles in the defensive response against salt treatment.

During salt stress, proteins involved in protein metabolism, such as leucine rich repeat containing protein kinase, aspartyl protease and proline rich family protein were up-regulated in the salt-tolerant PK faster than the salt-sensitive IR29. This indicates the salt-tolerant rice has salt-tolerant mechanisms in metabolism better than the salt-sensitive rice. This expression is similar to the expression of the transcription and translation proteins in PK and IR29.

The regulation of protein metabolism is related to the transporting protein in the reaction cell. The transporting proteins such as p125 protein, SEC-13 related protein, GTP binding protein, Opa-interacting protein and synaptobrevin-like protein were expressed in this study, but the expressions of these proteins were not different in salt-tolerant and salt-sensitive varieties. The up-regulation of these transporting proteins were found after 12 h of incubation with NaCl. This expression is similar to the expression of rice Rop gene *OsRacB* (subgroup of Rho family in plant GTP-binding protein) which is the potential accessory factor in plant salt tolerance (Nahm *et al.,* 2003). The rice Rop gene plays a role in the signal transduction network on the basis mechanism such as Ca^{2+} regulation, production of active oxygen and regulation of MAPK cascade. In addition, the rice *OsRab7* gene was differentially expressed in response to salt (Nahm *et al.*, 2003). The transgenic AtRab7 plants increased in the tolerance to salt and osmotic stresses and reduced an accumulation of reactive oxygen species during salt stress (Mazel *et al.,* 2004). The *smGTP* gene encoded a potentially protein which involved in signaling or vesicle transport to regulation of water-related stress responses, especially salt stress (Dombrowski, 2003).

In this study, the skeleton and cell organization protein (protein ABIl 1 and myosin heavy chain) were examined. The expression of myosin heavy chain was

increased under salt stress in the salt-tolerant and salt-sensitive varieties. As well as the study of Verma and Hong (2001) who reported that the myosin-like protein in rice root tip cells was up-regulated under salt stress. Myosin heavy chain is well known as a cytoskeleton-cell wall linker which usually accumulated at the plasma membrane and cell wall in diverse stress situations (Baluska *et al.*, 2003). The expression of protein ABIl 1 was shown in the ABA-evoked signalling which controlled the stomatal closure (Armstrong e*t al.*, 1995). This result evidences that the myosin-like protein and protein ABIl 1 of the salt-tolerant and salt-sensitive varieties sensed the stress signal through the cell wall when the rice cell exposed salt stress.

Moreover, the response proteins involved in the secondary metabolite biosynthesis such as NAD(P)H dependent deoxychalcone synthase and flavonol synthase were up-regulated only in the salt-sensitive IR29. This result is similar to the study of Whitehead *et al.* (1982) and Aoki *et al.* (2000) who found the phytoalexin which expressed after the plant exposed the stress.

Among 43 proteins, peroxidase, GST and NADH dehydrogenase were expressed as salt stress protein. The expression of peroxidase, GST, NADH dehydrogenase was found in the salt-tolerant PK, HJ and KDML 105 but not found in the salt-sensitive IR29 and PT1.

4. Expression analysis of salt stress response protein identified by enzymatic and proteomic analysis

Under salt induced oxidative stress, several cytotoxic reactive oxygen species (ROS) are continuously generated in cellular level such as the respiration. From this study, the proteomic analysis showed the decreasing of NADH dehydrogenase subunit 5. Sobhanian *et al.* (2010) reported that the increasing of ROS is related to the decreasing of NADH dehydrogenase subunit 5 activity. And then, ROS is eliminated by peroxidase which was detected in the enzyme assay. This result indicates the relationship between the enzymatic activity and proteomic assay in salt response.

CONCLUSION

1. This study suggests that the suspension cell culture is the particularly proper sample preparation technique for physiological, biochemical and molecular studies because of the high production of cell in a short time and the low quantity of uninteresting compositions which disturb in analysis step. The suitable phase of cell growth for analysis is found in the linear phase at the 6^{th}-9^{th} day after subculturing.

2. The salt stress response enzyme activities of β-glucosidase, peroxidase and ATPase in the salt-tolerant PK, HJ and KDML105 were different from the salt-sensitive IR29 and PT1. The up-regulation of peroxidase and ATPase was found after salt stress, while the expression of β-glucosidase which located in cytosol is not different between the salt stress and control treatment.

3. Forty three different salt stress-responsive proteins were identified by proteomic analysis. These protein which are involved in a wide range of cellular processes, *e.g.* transcription and translation process, protein metabolism, sugar metabolism, energy metabolism, secondary metabolite synthesis, hormone synthesis, glutathione metabolism, binding and transporting protein, cell defense and response protein, signal transduction and skeleton and cell organization. From KMC clustering, the different up-regulations of these proteins are found in the salt-tolerant and the salt-sensitive rice. Among 43 proteins, peroxidase, GST and NADH dehydrogenase subunit 5 were expressed as salt stress protein. The expression of peroxidase, GST and NADH dehydrogenase subunit 5 were found in the salt-tolerant PK, HJ and KDML 105 but not found in the salt-sensitive IR29 and PT1.

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APPENDICES

Appendix A

Media

Appendix Table A1 The composition of culture medium in Murashige and Skooge (1962) and Schenk and Hildebrandt (1972).

Component		MS	SH
Major salts		mg/l	mg/l
NH_4NO_3	(Ammonium nitrate)	1,650	-
KNO_3	(Potassium nitrate)	1,900	2500
$CaCl_2.2H_2O$	(Calcium chloride dehydrate)	440	200
$MgSO_4.7H_2O$	(Magnesium sulphate heptahydrate)	370	400
KH_2PO_4	(Potassium dihydrogen phosohate)	170	-
(NH4)H2PO4		-	300
Minor salts		mg/l	mg/l
KI	(Potassium iodide)	0.83	1
H_3BO_3	(Boric acid)	6.2	5
$MnSO_4.H_2O$	(Manganese sulphate monohydrate)	16.9	10
$ZnSO_4.7H_2O$	(Zinc sulphate heptahydrate)	8.6	1
$Na_2MoO_4.2H_2O$	(Sodium molybdate dehydrate)	0.25	0.1
$CuSO_4.5H_2O$	(Copper(II) sulphate pentahydrate)	0.025	0.2
$CoCl_2.6H_2O$	(Cobalt chloride hexahydrate)	0.025	0.1
NaFeEDTA	(Sodium ferrous EDTA)	37.25	20

Appendix Table A1 (Continued)

Component	MS	SH
Vitamins and organics	mg/l	mg/l
Myo-inositol	100	100
Nicotinic acid	0.5	0.5
Pyridoxin HCl	0.5	0.05
Thiamine HCl	0.1	0.5
Glycine	2	-

Appendix B

Enzyme activity analysis

1. Lowry's reagent preparation

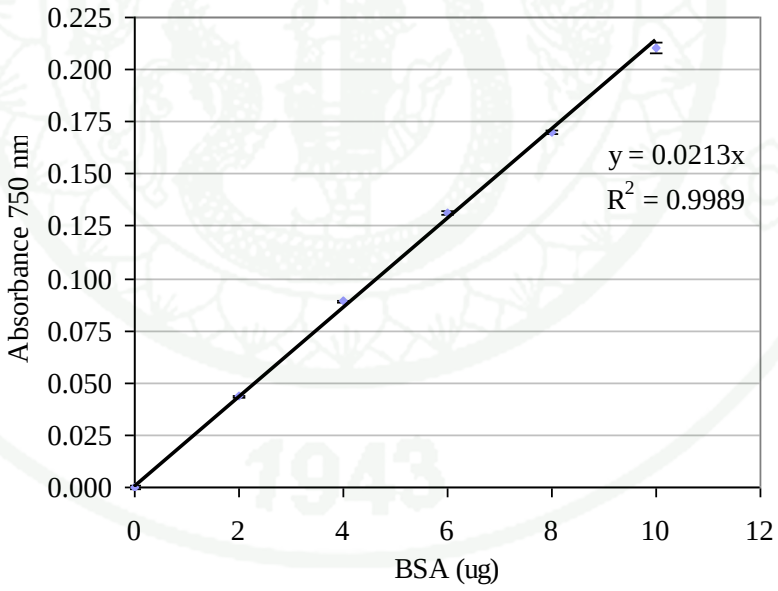
1.1 Lowry A

The 0.2 % CuSO4 and 0.4 % tartaric acid were mixed and slowly add with 20 % Na2CO3. Then the reagent was supplemented with 0.8 N NaOH and 5% SDS. The reagent was mixed and storaged in the dark.

1.2 Lowry B

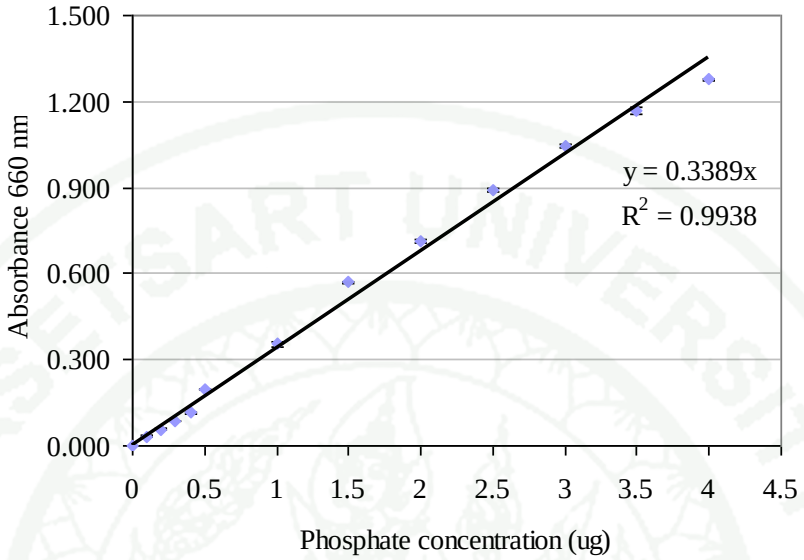
One volume of Folin-Ciocalteu phenol reagent was combined with 5 volume of sterile deionized water and the reagent was mixed and storaged in the dark.

2. Standard BSA



Appendix Figure B1 The standard curve of protein. The data was ploted absorbance at 750 nm as Y-axis and BSA concentration as X-axis. The data was represented the mean ± SD of 5 replicates.

3. Standard of inorganic phosphate

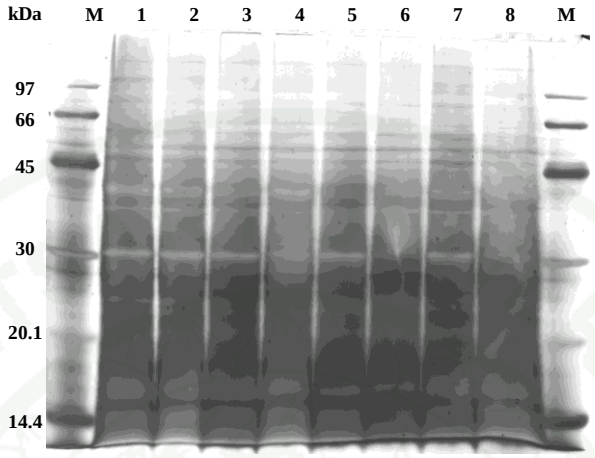


Appendix Figure B2 The standard curve of inorganic phosphate. The data was ploted absorbance at 660 nm as Y-axis and inorganic phosphate concentration (μg) as X-axis. The data was represented the mean ± SD of 5 replicates.

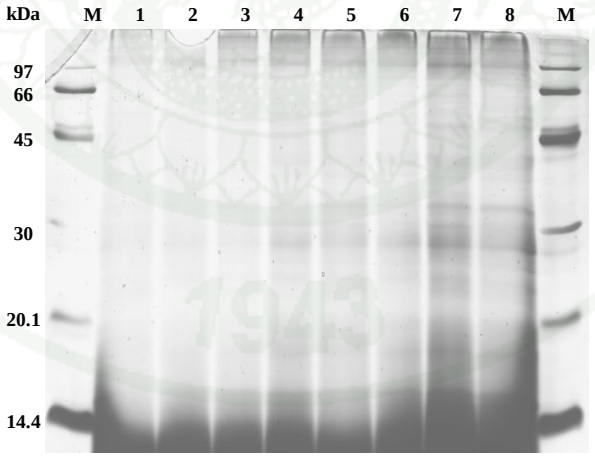
Appendix C

Salt responding protein analysis

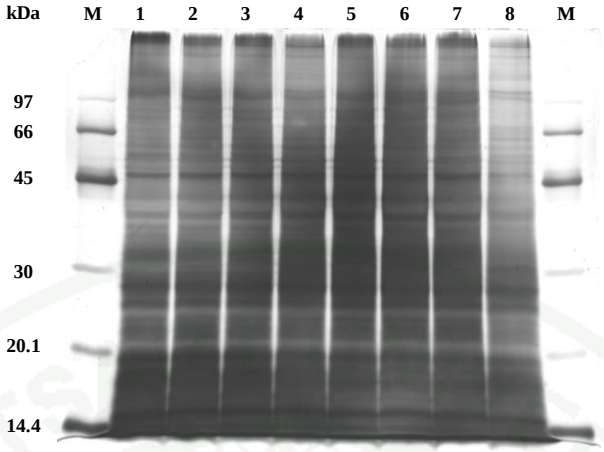
1. 1D SDS-PAGE and visualized by silver staining



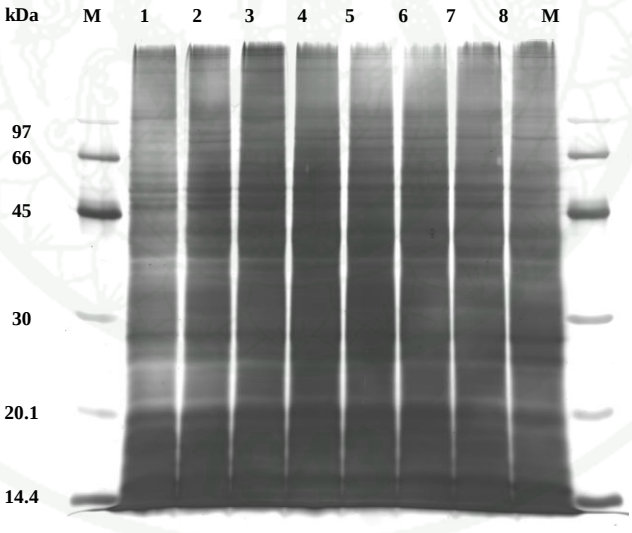
Appendix Figure C1 A 12 .5% SDS-PAGE showing expression patterns of Pokkali of control group at 0, 1, 3, 6 h (lanes 1, 3, 5, 7) and treatment group at 0, 1, 3, 6 h (lanes 2, 4, 6, 8). Lanes M is the protein marker.



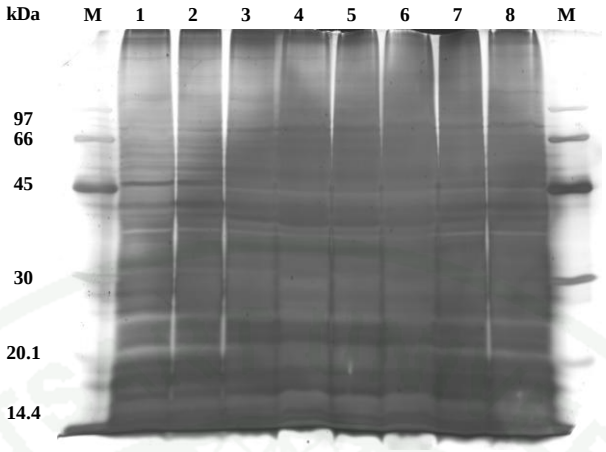
Appendix Figure C2 A 12 .5% SDS-PAGE showing expression patterns of Pokkali of control group at 12, 24, 48, 72 h (lanes 1, 3, 5, 7) and treatment group at 12, 24, 48, 72 h (lanes 2, 4, 6, 8). Lanes M is the protein marker.



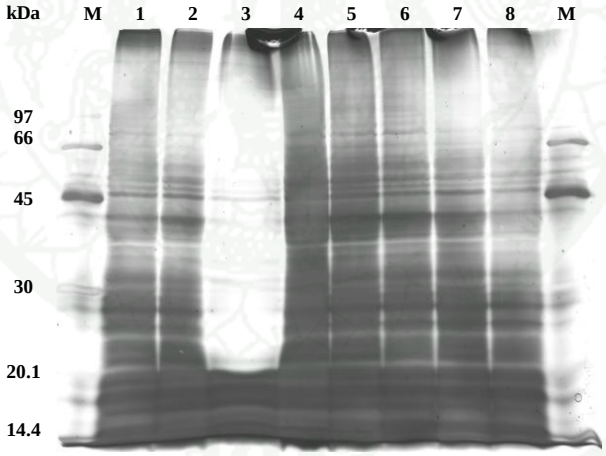
Appendix Figure C3 A 12 .5% SDS-PAGE showing expression patterns of IR29 of control group at 0, 1, 3, 6 h (lanes 1, 3, 5, 7) and treatment group at 0, 1, 3, 6 h (lanes 2, 4, 6, 8). Lanes M is the protein marker.



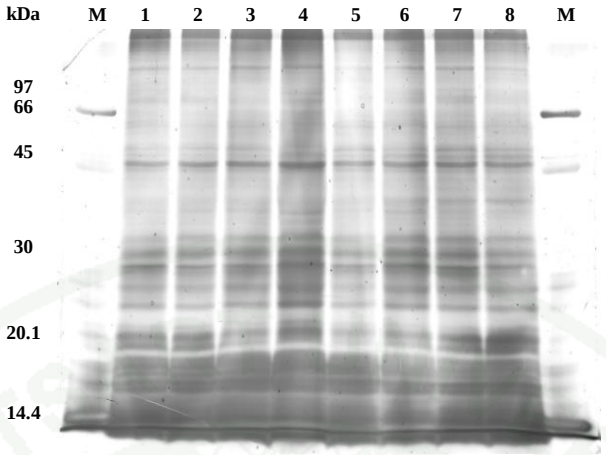
Appendix Figure C4 A 12 .5% SDS-PAGE showing expression patterns of IR29 of control group at 12, 24, 48, 72 h (lanes 1, 3, 5, 7) and treatment group at 12, 24, 48, 72 h (lanes 2, 4, 6, 8). Lanes M is the protein marker.



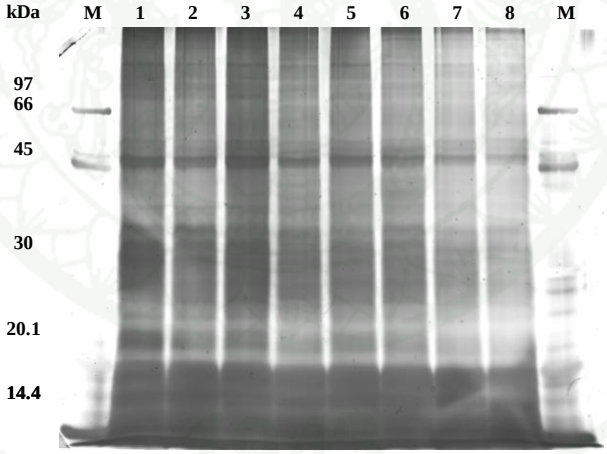
Appendix Figure C5 A 12 .5% SDS-PAGE showing expression patterns of HJ of control group at 0, 1, 3, 6 h (lanes 1, 3, 5, 7) and treatment group at 0, 1, 3, 6 h (lanes 2, 4, 6, 8). Lanes M is the protein marker.



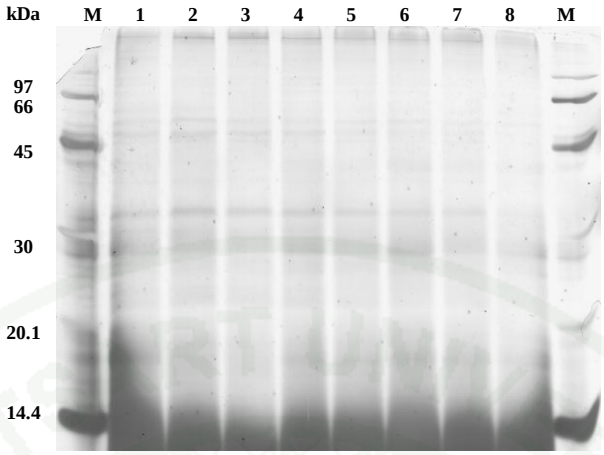
Appendix Figure C6 A 12 .5% SDS-PAGE showing expression patterns of HJ of control group at 12, 24, 48, 72 h (lanes 1, 3, 5, 7) and treatment group at 12, 24, 48, 72 h (lanes 2, 4, 6, 8). Lanes M is the protein marker.



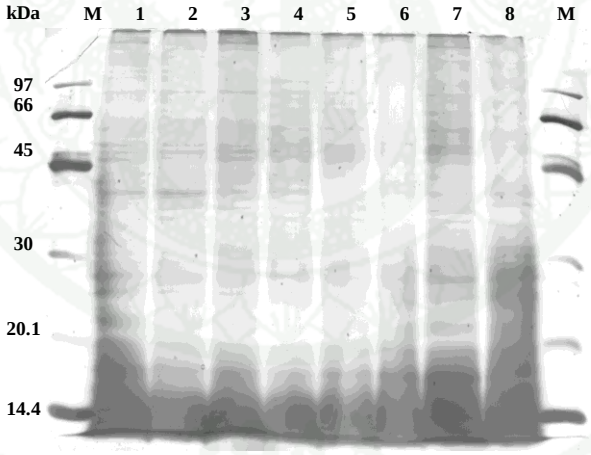
Appendix Figure C7 A 12 .5% SDS-PAGE showing expression patterns of KDML105 of control group at 0, 1, 3, 6 h (lanes 1, 3, 5, 7) and treatment group at 0, 1, 3, 6 h (lanes 2, 4, 6, 8). Lanes M is the protein marker.



Appendix Figure C8 A 12 .5% SDS-PAGE showing expression patterns of KDML105 of control group at 12, 24, 48, 72 h (lanes 1, 3, 5, 7) and treatment group at 12, 24, 48, 72 h (lanes 2, 4, 6, 8). Lanes M is the protein marker.



Appendix Figure C9 A 12 .5% SDS-PAGE showing expression patterns of PT1 of control group at 0, 1, 3, 6 h (lanes 1, 3, 5, 7) and treatment group at 0, 1, 3, 6 h (lanes 2, 4, 6, 8). Lanes M is the protein marker.



Appendix Figure C10 A 12 .5% SDS-PAGE showing expression patterns of PT1 of control group at 12, 24, 48, 72 h (lanes 1, 3, 5, 7) and treatment group at 12, 24, 48, 72 h (lanes 2, 4, 6, 8). Lanes M is the protein marker.

2. Totally salt responding proteins

Appendix Table C1 Totally differential expressed proteins in rice cell suspension under salt stress by analyzed with DeCyderMS Differential Analysis software.

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	Pokkali								IR29							
					0	1	3	6	12	24	48	72	0	1	3	6	12	24	48	72
Skeleton and cell organization																				
Extensin-like protein	CA753366	R.GQRGYSXXRS.-	1/17	29486	2.2	1.1	-5.2	0.1	-0.1	3.3	-5.2	-5.7	-2.1	-1.2	1.7	-0.5	18.8	-18.0	-0.1	-1.3
Myosin heavy chain	gi|31193918	R.LMINNR.I + Oxidation (M)	25/30	170023	18.9	-22.3	-20.6	18.6	-0.7	0.0	17.2	16.2	17.9	-19.6	-0.8	-3.2	2.7	0.0	0.0	22.8
Protein ABIL1	gi|255541748	K.AGPVS.-	1/27	34537	-18.7	-3.2	-0.6	-3.1	-0.5	-1.7	17.6	0.0	0.8	0.4	-1.1	-5.4	-0.6	-0.3	3.2	-2.7
Zeatin O-glucosyltransferase	CA764857	R.TLTLGQGSR.S	1/12	25912	-1.7	-0.1	-1.1	0.0	0.6	1.3	-0.1	-1.3	0.0	-1.2	0.6	-1.6	1.1	-0.6	-0.4	-0.3
Energy metabolism																				
Aacyl-coa synthetase	BE041069	R.GPTSSGGSR.H	1/11	25215	0.4	-1.0	1.8	-1.1	2.5	-2.7	2.4	1.6	1.2	-0.8	3.2	-0.1	0.6	1.1	-0.2	-1.9
beta-hydroxyacyl-acp dehydratase	gi|8334683	-.LQGIP.-	1/6	36394	-17.0	-4.6	-3.4	3.0	3.3	0.2	13.6	-17.4	1.3	-1.9	-0.7	1.2	-1.9	-0.9	-1.0	-0.4
NADH dehydrogenase subunit 5	CA756211	R.ELNGN.-	1/3	21618	-0.7	-19.4	1.0	1.5	1.5	-1.2	-3.2	1.1	-2.0	-0.5	-0.9	2.8	1.7	-1.3	0.4	-16.8
Phosphoribosylamidoimidazole-succinocarboxamide synthase precursor	CA764505	R.KCYIXG.-	2/16	28122	-1.2	0.9	3.3	0.4	1.5	-3.8	-4.3	0.5	0.0	-1.1	4.8	-0.9	3.3	-3.2	0.1	-2.8

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	Pokkali								IR29							
					0	1	3	6	12	24	48	72	0	1	3	6	12	24	48	72
3-oxoacyl-[acyl-carrier-protein] synthase i	gi|8335079	-.PASFV.-	1/5	94375	-0.2	-1.9	-4.1	0.3	-1.2	3.9	-0.3	-1.4	2.7	-0.5	2.9	0.0	-1.4	1.3	0.0	-0.9
Reductase	gi|27547747	R.RHGAS.-	1/6	81625	-0.8	-0.9	-0.9	-0.6	0.4	-0.4	3.9	0.4	1.1	-2.2	4.1	-3.6	0.5	0.1	-0.1	1.8
Protein metabolism																				
Aminotransferase	gi|25797636	R.HHRGGGR.T	1/19	10076	-0.9	0.3	-13.5	4.1	-0.2	0.9	-1.8	-18.7	1.3	-0.1	0.9	-3.1	-0.6	1.6	-2.2	-1.2
Leucine rich repeat containing protein kinase	gi|48716959	K.TDYESNLTVQGTQQT.-	1/6	114163	-0.7	1.2	-1.9	-4.7	-1.3	-3.9	-3.3	-1.0	-0.8	-4.5	3.1	-1.3	-1.2	3.3	1.1	2.6
Proline-rich family protein	gi|18398103	R.SKHGMFGGK.R + Oxidation (M)	1/18	18383	14.1	-3.4	-5.5	-18.7	-1.4	1.1	-4.5	0.0	13.2	2.8	0.0	12.2	2.0	-0.2	-0.7	0.7
Protease	gi|25801939	K.TPGDS.-	6/17	79816	4.6	0.2	-1.0	-2.6	1.1	-2.0	-0.7	4.9	-0.2	3.6	1.1	3.7	2.2	-0.4	1.5	0.7
Ubiquitin protein	CA759059	R.DSIGKKK.K	8/27	8815	0.0	-2.0	-1.4	-0.5	-0.1	-3.6	1.0	-2.3	0.1	-0.9	1.1	-0.6	0.2	5.4	0.3	-0.2
Secondary metabolite metabolism																				
Flavonol 4'-sulfotransferase	gi|27547220	-.KPCMXKR.X + Oxidation (M)	1/9	67582	0.4	2.1	-2.3	-3.0	-1.6	0.4	-0.7	1.5	1.3	-1.9	3.2	1.7	1.1	-1.0	0.1	1.0
NAD(P)H dependent 6'-deoxychalcone synthase	gi|27921023	K.MLYFLF.- + Oxidation (M)	1/4	79838	0.7	3.6	0.8	-1.3	-1.2	1.1	-0.1	-1.7	0.4	-0.4	3.3	0.9	0.2	1.4	-0.6	3.2
Os09g0368200	gi|255678841	R.YRLFMTLK.L	1/1	62278	-15.8	0.0	13.4	17.4	0.0	0.0	0.0	0.0	-15.6	-3.7	-14.5	-0.1	-1.6	-2.6	-0.8	0.0

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	Pokkali								IR29							
					0	1	3	6	12	24	48	72	0	1	3	6	12	24	48	72
Sugar metabolism																				
Alcohol dehydrogenase 1	gi|20165	K.GSTVAIFGLGAVGLAAAEGAR.I	2/98	41567	-3.3	-2.2	4.7	2.9	14.0	-0.4	1.0	2.9	0.0	-1.0	0.0	16.7	-5.7	17.5	4.0	-0.1
Aldolase C-1	gi|786178	K.VSPQLIAEYTVR.A	7/137	39141	-2.2	2.1	-18.7	0.0	0.0	0.0	18.3	-2.4	-2.5	16.1	-2.6	0.0	-17.3	19.5	-17.6	0.7
Beta-glucosidase					0.2	-0.1	0.2	-0.4	0.1	0.0	0.1	-0.2	-1.0	0.0	0.2	-0.5	0.1	-0.6	0.1	-0.1
Glyceraldehyde-3-phosphate dehydrogenase	gi|19880027	R.IAWEEPFGPVLPVIR.I	3/73	54856	-0.8	1.6	-1.9	1.0	0.2	0.4	-3.5	-0.6	1.2	0.2	1.0	0.8	0.0	-0.2	0.5	-18.5
Glycogenin glucosyltransferase	gi|8335675	-.PTKGL.-	1/9	9078	-18.3	-2.6	2.6	0.0	-16.6	0.0	18.6	0.0	-3.7	13.9	1.3	13.6	-2.5	-5.6	1.0	-1.2
Glycosyl transferase ypfP	gi|27547464	K.GSASPWQAAAGACGCPSRCR.T	1/5	29011	15.6	-13.7	4.7	-16.6	-18.1	18.6	18.2	-3.5	18.4	-1.1	-12.9	-18.9	-18.5	18.8	18.1	-18.0
Histidinol dehydrogenase	CA763212	-.SFFQA.-	1/10	26061	3.0	-1.3	0.7	0.6	-0.5	3.2	-0.8	-2.7	1.6	-1.4	4.7	-2.0	-3.1	2.9	-2.8	2.1
Sucrose synthase	gi|20374	K.SIGNGVQFLNR.H	7/60	92568	0.5	1.8	-17.8	-2.1	-0.1	-20.0	-4.6	-15.1	0.6	0.1	-0.5	-5.5	-1.9	-1.3	1.0	-1.2
Signal transduction																				
Ankyrin-like protein	BU099192	R.ELGPGL.-	1/16	12208	1.7	-1.2	-22.1	-1.2	0.6	-5.9	-0.2	-2.2	-4.1	-3.4	-0.6	4.6	1.3	-0.4	2.3	-0.1
Serine/threonine-specific protein kinase	gi|50251369	K.KEVDGESSKR.R	3/22	84451	0.4	-0.6	0.3	-0.5	0.1	2.2	-3.8	-3.8	-0.9	-0.2	-1.4	-1.8	1.5	0.4	1.7	-0.1
OSK3	gi|25806829	R.FFPAIMNK.G	1/1	22090	14.4	-18.2	-15.2	19.6	-18.2	21.1	-19.1	20.0	0.0	-19.3	0.0	0.0	0.0	0.0	0.0	17.4

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	Pokkali								IR29							
					0	1	3	6	12	24	48	72	0	1	3	6	12	24	48	72
Transcription																				
CAA303717.1 protein	gi|5777629	R.RSSDLLNAADDDQGFAADA.-	2/56	37205	-2.4	-0.1	0.7	4.6	-1.3	0.9	-1.5	-0.5	-0.6	-1.7	-0.6	-2.7	-2.1	0.1	-1.5	-1.7
dbp-5 protein	gi|8334862	R.MLQLGGPR.Q + Oxidation (M)	1/11	35216	0.9	14.8	0.0	17.1	0.3	0.0	13.8	0.0	0.3	2.8	0.9	-0.1	0.8	-0.3	-1.2	-5.0
Em binding protein-1a	CA757353	-.LETAH.-	1/8	22861	-19.8	-2.3	-16.2	0.3	-4.2	0.8	-1.8	14.2	0.6	-1.5	-2.8	-4.3	3.5	-0.9	17.5	-1.5
F-box domain containing protein	gi|77556383	.GLGLCGCAINHGVISR.A	1/1	67301	-0.7	1.2	-1.9	-4.7	-1.3	-3.9	-3.3	-1.0	-0.8	-4.5	3.1	-1.3	-1.2	3.3	1.1	2.6
F2K11.18	gi|25803017	-.PSAQT.-	1/9	25432	-16.7	1.3	4.5	-6.8	1.1	-14.8	0.0	0.0	-15.8	14.7	-17.1	17.4	3.4	-1.2	-0.2	2.5
Gibberellin-stimulated transcript 1 like protein	gi|25801810	R.TAPTARSR.R	1/22	54417	3.5	6.0	-2.9	4.1	3.7	-3.5	-1.5	-0.3	1.5	4.8	-1.8	2.0	3.8	4.3	2.7	1.7
HARP protein	gi|25803016	K.TLRNVRT.-	1/1	67612	3.5	6.0	-2.9	4.1	3.7	-3.5	-1.5	-0.3	1.5	4.8	-1.8	2.0	3.8	4.3	2.7	1.7
HD-Zip protein	gi|25802926	R.YAFTKVVFI.-	1/3	78151	-2.2	-0.2	-1.1	1.8	-0.5	0.6	0.4	0.5	4.2	-0.6	-0.7	1.9	-0.9	-1.1	-0.3	1.1
Homeodomain leucine zipper protein	gi|25799785	-.LRPNII.-	1/9	22392	-0.1	4.4	0.8	-0.8	1.9	3.6	-5.3	-17.3	-1.4	-0.5	-2.1	-0.7	0.7	-0.9	-2.8	-0.2
Integrase core domain	gi|62733246	K.AYIEKVLMK.F + Oxidation (M)	1/6	115339	1.1	0.0	-0.8	-18.1	-18.0	18.8	0.2	-17.6	17.1	-14.9	-17.3	16.2	5.0	18.1	-0.2	3.0
Lysophospholipase homolog	CA760360	-.FCMAQLSQ.- + Oxidation (M)	1/7	26565	0.5	1.4	-3.8	0.3	-4.5	-3.2	0.1	-0.4	-4.6	5.1	-1.7	-19.5	17.8	-1.1	2.6	-15.2
Opa-interacting protein	gi|25806125	R.KEEASDY.-	1/9	25333	0.0	-3.0	4.5	-4.3	0.0	3.6	15.2	0.0	0.0	-19.5	0.0	13.8	-19.1	0.0	0.0	12.6
Origin recognition complex subunit 2	CA759483	-.RSESPLMAK.I + Oxidation (M)	1/8	25328	22.1	-2.6	1.6	0.1	-0.4	2.4	15.7	2.0	-1.1	0.7	-19.4	-17.6	-1.0	19.6	-19.8	18.2

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	Pokkali								IR29							
					0	1	3	6	12	24	48	72	0	1	3	6	12	24	48	72
PCNA	CA756267	-.LCMNSLYR.E + Oxidation (M)	1/17	24159	1.0	5.3	1.9	0.0	-0.6	-17.0	3.6	15.1	0.6	0.0	-20.6	0.0	20.0	-19.8	-19.3	0.0
Phytepsin precursor	CA756102	R.TXSESGRSTQSRSSTWPTAPR.G	2/6	23116	0.7	-3.4	5.4	0.9	2.7	-1.9	3.8	0.5	4.0	1.0	-0.6	-2.0	0.2	1.0	-0.8	2.4
P0432B10.4	gi|20161462	R.FAVKALMKR.R	1/7	49353	0.5	-17.5	14.0	17.6	17.7	16.4	0.0	16.3	-0.9	-15.2	-16.0	-3.2	1.7	-15.8	14.1	0.1
psi-h precursor	BE040299	-.LSSNNGASS.-	1/5	22334	1.6	0.4	1.1	2.1	-0.5	0.3	-1.9	-0.9	1.6	0.8	-1.8	-0.9	1.9	0.6	1.2	-3.1
RNA binding protein	gi|8335896	-.LEGVVGLGL.-	1/7	80312	-2.8	1.0	-4.9	3.1	-1.5	-1.6	-5.6	-2.9	4.8	-1.8	3.3	2.6	0.6	-4.2	-0.8	-16.3
Slbp-P1	CA762496	R.EGTATFA.-	1/10	25778	0.0	-14.3	0.0	0.0	0.0	-16.0	0.0	0.0	14.0	0.0	16.9	0.0	-14.7	-13.1	0.0	-14.3
Transcription factor	gi|25803301	K.ALGSH.-	1/10	62034	0.5	2.2	-1.9	1.9	14.5	1.0	-3.7	-1.4	-0.3	-3.5	2.6	-1.8	0.4	0.0	2.7	4.0
Unclassified retrotransposon protein	gi|242117496	K.WSLARNFK.A	4/42	145798	1.4	1.0	0.0	-13.6	15.7	-14.7	13.4	-17.3	-2.2	2.7	-1.8	0.2	14.0	1.8	-2.2	2.8
zinc finger, DHHC domain containing 5	CA753613	-.IGYSGGLK.S	1/10	8857	3.3	0.7	-0.5	-0.5	-0.2	2.7	-0.1	-2.3	-0.3	0.3	-1.1	-0.6	0.7	-0.9	0.2	-2.4
Translation																				
Aspartyl protease	gi|25797766	K.KKNRMK.Q	1/17	22163	-2.4	0.3	-1.0	1.6	-0.5	-16.3	-5.2	-17.2	0.1	-1.6	2.5	-1.2	-2.2	-2.3	2.2	-0.1
Cytochrome c oxidase subunit III	CA762510	K.AQKNEMK.K	2/6	25411	1.3	-1.6	0.8	1.2	-0.7	-0.1	-0.9	-2.0	-0.5	2.3	0.8	-3.9	5.5	3.2	2.4	-1.6
Elongation factor ef-2	BE040066	R.HMRAG.- + Oxidation (M)	1/2	35389	0.0	0.0	17.8	0.0	0.0	-15.7	0.0	0.0	16.5	0.0	13.8	-16.2	17.9	17.3	17.4	-14.5
40S ribosomal protein S15	CA758185	R.ATAFPNP.-	1/7	32243	-1.8	1.8	-0.8	1.5	-1.6	-1.1	-2.2	-2.5	1.1	0.8	-3.0	1.0	1.1	0.2	-0.5	-0.1

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	Pokkali								IR29							
					0	1	3	6	12	24	48	72	0	1	3	6	12	24	48	72
60s ribosomal protein l27	BE040610	R.VLADHLLR.V	2/11	33450	1.4	-0.7	0.0	-0.1	1.7	-0.3	0.4	-3.4	-0.9	0.1	-1.9	-1.7	0.0	0.3	-0.4	0.3
60s ribosomal protein l6	gi|8334650	R.RPGSH.-	1/5	21429	2.9	16.4	0.0	-14.1	0.0	0.0	14.1	0.0	16.7	0.0	12.7	16.6	14.1	-13.4	12.3	2.2
60S ribosomal protein L24	CA756401	R.LNVDIFD.-	1/4	23148	-1.7	0.7	-1.1	5.2	1.3	-15.6	-0.8	0.1	3.7	0.8	-1.4	-18.3	0.2	-1.8	2.2	1.1
Translation elongation factor EF-1 alpha	CA753016	K.RGLEX.-	1/6	25917	1.7	-1.2	-22.1	-1.2	0.6	-5.9	-0.2	-2.2	-4.1	-3.4	-0.6	4.6	1.3	-0.4	2.3	-0.1
Translation initiation factor eIF-6	CA763564	R.SPPCLGTGH.-	1/3	26761	-2.3	0.6	2.4	4.1	-0.7	-18.2	17.5	-19.0	-1.7	0.0	-1.1	0.5	-15.8	-2.1	0.1	-15.6
von hippel-lindau binding protein 1	BE040150	K.IVPSSGRS.-	1/4	34671	0.0	-20.3	-18.7	18.3	-1.6	0.0	-1.4	-1.6	1.9	17.3	0.9	-17.3	-3.7	-19.1	-17.6	0.5
Cell defense and rescue system																				
ABC transporter ATP-binding protein	gi|25806938	-.ASLSTFR.G	1/12	64258	0.4	2.9	-0.8	-0.2	2.6	3.7	0.4	0.6	0.4	0.3	2.1	0.7	2.3	1.4	-2.7	3.0
Aquaporin	gi|8334728	-.IKGCIM.-	1/1	100816	-2.1	0.1	-1.5	-2.0	2.4	0.3	0.2	1.5	0.3	-2.7	2.4	0.6	-0.8	0.0	-0.1	-0.9
Basidic peroxidase					-21.6	-1.2	25.2	26.2	4.0	7.9	-10.5	-41.7	-4.8	6.1	-7.6	-8.7	2.8	-1.0	14.1	21.0
Cyclophilin	gi|8335257	R.SFPVLPL.-	1/6	76662	2.1	-3.8	-2.0	0.9	5.2	-3.8	-7.0	0.3	-2.8	-3.4	5.5	2.8	0.8	-0.5	0.9	-16.6
Cytochrome P450 71D8	CA765579	R.CSSRNGFTVK.R	1/15	29857	-0.4	-0.7	-1.9	-0.1	0.7	0.1	0.6	-2.2	1.2	-0.7	1.3	-1.6	-1.4	-1.6	0.4	0.9
Enolase	gi|780372	K.VNQIGSVTESIEAVK.M	4/54	48299	-0.1	0.4	-2.0	4.3	-2.2	1.4	-2.2	-0.4	-2.7	1.0	-0.7	-6.4	-0.7	-1.7	-3.5	-1.7

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	Pokkali								IR29							
					0	1	3	6	12	24	48	72	0	1	3	6	12	24	48	72
Glutathione s-transferase	gi|25798958	K.KKSTAGGR.S	2/12	22473	-19.2	0.0	16.8	-2.1	-16.8	-2.4	0.6	-17.2	-17.8	-0.3	0.0	15.2	16.8	-16.6	1.2	-17.9
HUMMDR1 Human P-glycoprotein	gi|2796454	K.GGPXL.-	1/2	5621	-1.4	0.0	-1.4	2.2	1.8	-15.2	16.7	0.0	0.1	-3.8	-13.3	13.8	-2.5	1.2	-0.3	-3.6
Hypersensitivity-induced response-like protein	gi|25803492	K.NSMLLAK.Q + Oxidation (M)	1/9	25374	-15.0	-18.4	19.3	0.0	0.0	-19.8	-21.1	0.0	0.0	0.0	-18.0	0.0	0.0	0.0	0.0	0.0
Iron receptor	gi|25798451	K.GARAGTVAR.R	1/6	18003	-17.1	0.0	0.0	0.0	0.0	0.0	0.0	-16.8	16.8	-16.8	18.0	17.7	0.0	0.0	-3.1	14.6
Manganese superoxide dismutase	gi|601869	K.NVRPDYLSNIWK.V	1/31	24932	1.1	-1.4	-2.3	1.1	-1.0	-1.5	-0.4	0.1	1.5	-1.1	-4.6	-1.5	0.3	-1.5	-0.3	-1.3
Metallothionein-like protein	gi|2800520	R.GGXXP.-	1/2	47125	2.1	1.1	-0.7	-0.2	0.3	0.0	-0.1	-3.1	-0.2	1.7	2.1	1.1	0.0	-0.3	-2.0	-14.5
Neutral peroxidase					-16.6	1.9	-4.2	14.2	-7.7	-3.7	-20.1	-49.0	-3.2	4.6	-14.2	-10.2	4.5	-1.6	17.2	22.2
OJ000114_01.12	gi|39545657	R.GSGNGFEGLK.T	1/22	40790	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pathogenesis-related protein PR-10b	CA759435	R.RAXRLGVGGAGVEGLSDAPAMPK.V	1/11	24168	3.8	-3.1	2.4	-4.2	-1.4	0.2	-3.6	0.2	3.4	-2.0	5.1	2.1	-0.6	0.9	3.4	-3.1
Phenylalanine ammonia-lyase	gi|8336796	K.HLVALLAGELK.L	1/13	69395	1.0	3.6	-4.2	-1.6	2.1	-0.6	-18.6	19.2	19.1	17.0	18.4	-4.1	0.0	-1.9	16.0	-15.3
Phosphatidylinositol-4-phosphate-5-kinase	gi|25803548	R.YTSIQR.M	1/21	69009	4.5	-1.3	-3.8	2.0	0.0	-1.4	15.5	0.6	0.6	1.1	5.6	3.2	0.6	-0.7	-0.7	-14.2

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	Pokkali								IR29							
					0	1	3	6	12	24	48	72	0	1	3	6	12	24	48	72
Plasma membrane ATPase					0.0	0.1	0.1	-0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	-0.1	0.1	-0.1
Retroelement	gi|19881553	R.GKAGELGVGAPR.D	1/2	92534	1.1	0.0	-0.8	-18.1	-18.0	18.8	0.2	-17.6	17.1	-14.9	-17.3	16.2	5.0	18.1	-0.2	3.0
Salivary proline-rich glycoprotein precursor	gi|8334645	R.ADKAKQAG.-	1/9	104377	-3.0	0.4	-0.4	3.3	1.1	-2.6	0.4	2.8	2.4	2.2	-0.1	-2.5	1.4	1.0	1.0	3.8
Total ATPase					0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Transporting																				
Brefeldin A-sensitive Golgi protein - like	CA762643	K.SSLMSFKK.K	1/11	20874	-0.4	0.9	-1.3	0.2	1.1	-1.4	-0.6	-1.0	1.5	-0.4	-0.8	-4.1	2.2	-0.1	-0.2	2.3
GTP-binding protein	CA757475	K.MQIQLH.- + Oxidation (M)	1/17	23646	-1.2	0.9	3.3	0.4	1.5	-3.8	-4.3	0.5	0.0	-1.1	4.8	-0.9	3.3	-3.2	0.1	-2.8
Multispanning membrane protein	BE040128	-.SSIDLNF.-	1/6	37181	3.3	0.7	-0.5	-0.5	-0.2	2.7	-0.1	-2.3	-0.3	0.3	-1.1	-0.6	0.7	-0.9	0.2	-2.4
SEC13-related protein	CA754964	-.YRFTIS.-	1/16	23420	-1.2	0.9	3.3	0.4	1.5	-3.8	-4.3	0.5	0.0	-1.1	4.8	-0.9	3.3	-3.2	0.1	-2.8
Secretory carrier membrane protein	gi|25803448	K.GFGGIHK.G	1/5	69328	0.6	-0.6	-2.1	-0.8	0.5	-2.8	-2.4	-1.0	1.2	0.6	1.5	0.2	0.8	2.1	0.6	-18.2
p125 protein	gi|25799240	R.CPTGSPR.R	1/7	23044	-0.9	0.3	-13.5	4.1	-0.2	0.9	-1.8	-18.7	1.3	-0.1	0.9	-3.1	-0.6	1.6	-2.2	-1.2
Synaptobrevin-like protein	BE040833	R.ADAGGGGGGR.R	1/27	30595	18.9	-22.3	-20.6	18.6	-0.7	0.0	17.2	16.2	17.9	-19.6	-0.8	-3.2	2.7	0.0	0.0	22.8
Storage protein																				
Preproglutelin	AA752620	-.MXCCSSLY.- + Oxidation (M)	1/18	16976	22.9	5.6	3.4	-0.1	0.0	0.0	0.0	16.8	0.0	-20.3	0.0	0.0	0.0	0.0	0.0	0.0

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	Pokkali								IR29							
					0	1	3	6	12	24	48	72	0	1	3	6	12	24	48	72
Prolamin PPROL 14 precursor	CA762870	-.ADPRGIVPTVPPFSR.K	1/13	24031	0.4	-1.0	1.8	-1.1	2.5	-2.7	2.4	1.6	1.2	-0.8	3.2	-0.1	0.6	1.1	-0.2	-1.9
DNA processing																				
mutM homologue-1	gi|25806579	R.QGKMVVAR.R	1/11	26107	-15.1	-17.3	4.1	-0.1	0.7	4.2	-1.3	0.2	16.6	-16.8	0.0	13.9	-3.5	18.6	0.5	18.0
Nucleoside diphosphate kinase	AA752790	K.NGXPKIK.N	1/10	26566	3.1	0.4	-0.8	-4.1	-2.4	1.4	0.3	2.6	-1.2	-1.4	-1.0	-2.5	-1.7	2.2	-2.6	-0.4
Ribonucleosie reductase 1 (Large chain)	gi|25806543	-.LQGLP.-	1/6	26009	-17.0	-4.6	-3.4	3.0	3.3	0.2	13.6	-17.4	1.3	-1.9	-0.7	1.2	-1.9	-0.9	-1.0	-0.4
Unknown																				
agCP12256	CA757055	-.VWELIQILKIPKE.-	1/12	20801	0.4	-1.0	1.8	-1.1	2.5	-2.7	2.4	1.6	1.2	-0.8	3.2	-0.1	0.6	1.1	-0.2	-1.9
agCP5654	gi|25803664	R.LFENKY.-	1/12	26289	-14.3	-3.1	-1.2	-5.3	3.9	-1.8	-4.2	0.2	5.8	1.5	-0.9	-3.0	-2.9	0.1	0.0	-0.2
agCP8018	CA759626	R.AVVLSR.Q	2/31	26861	-0.6	-0.7	3.5	-0.4	-0.8	3.3	-18.4	-0.7	1.1	-0.7	-1.1	-0.4	0.4	-1.3	1.2	-1.6
mtn12	BE040684	-.RLILHPPH.-	1/4	39754	2.3	0.0	-1.6	0.9	-0.6	0.0	0.2	-0.6	1.7	-1.0	-0.4	1.1	-0.2	-0.4	-0.3	-0.8
OSJNBa0014K14.4	gi|70663918	M.APPLLPRGAALLLLLR.S	1/1	68298	-0.7	1.2	-1.9	-4.7	-1.3	-3.9	-3.3	-1.0	-0.8	-4.5	3.1	-1.3	-1.2	3.3	1.1	2.6
OSJNBa0029C04.3	gi|25797412	R.DTESR.A	1/5	83946	-1.8	-1.9	0.1	-4.6	-2.7	0.6	-1.9	-4.4	0.9	-6.1	0.6	0.5	1.9	3.2	0.9	1.7
OSJNBa0038O10.8	gi|38344436	R.RSSPGLVAAPR.L	2/11	56352	1.1	0.0	-0.8	-18.1	-18.0	18.8	0.2	-17.6	17.1	-14.9	-17.3	16.2	5.0	18.1	-0.2	3.0

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	HJ								KDML105							
					0	1	3	6	12	24	48	72	0	1	3	6	12	24	48	72
Skeleton and cell organization																				
Extensin-like protein	CA753366	R.GQRGYSXXRS.-	1/17	29486	-0.5	18.3	-3.0	0.0	-0.7	-16.5	0.0	0.0	-0.3	-1.0	1.2	0.0	-3.9	-0.2	2.0	-0.4
Myosin heavy chain	gi|31193918	R.LMINNR.I + Oxidation (M)	25/30	170023	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0	17.3
Protein ABIL1	gi|255541748	K.AGPVS.-	1/27	34537	-1.3	-17.2	0.0	-5.0	14.6	0.0	12.5	-1.9	0.3	0.0	15.6	14.4	0.1	-15.8	-17.8	-1.9
Zeatin O-glucosyltransferase	CA764857	R.TLTLGQGSR.S	1/12	25912	-0.2	0.1	-0.9	2.2	2.0	0.6	-1.7	-0.9	-2.2	1.1	0.2	0.1	-1.4	0.0	3.8	1.2
Energy metabolism																				
Aacyl-coa synthetase	BE041069	R.GPTSSGGSR.H	1/11	25215	-2.0	2.4	-0.4	-1.2	0.8	-1.3	0.1	-0.4	1.6	1.6	0.1	0.4	-0.9	-1.1	0.2	1.2
beta-hydroxyacyl-acp dehydratase	gi|8334683	-.LQGIP.-	1/6	36394	2.7	4.4	0.0	1.1	-1.6	3.4	-3.1	-0.6	15.5	15.3	-15.2	14.4	-0.8	2.8	-1.0	2.0
NADH dehydrogenase subunit 5	CA756211	R.ELNGN.-	1/3	21618	-14.8	-1.0	-18.1	2.6	-1.5	17.7	0.1	0.0	-18.7	-16.6	-2.8	0.2	-1.3	-0.6	-17.8	-0.8
Phosphoribosylamidoimidazole-succinocarboxamide synthase precursor	CA764505	R.KCYIXG.-	2/16	28122	-0.5	1.9	-0.7	-0.7	0.6	-0.6	0.3	-1.7	-0.3	-1.8	-1.1	1.1	0.0	-2.3	1.0	-0.5

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	HJ								KDML105							
					0	1	3	6	12	24	48	72	0	1	3	6	12	24	48	72
3-oxoacyl-[acyl-carrier-protein] synthase i	gi|8335079	-.PASFV.-	1/5	94375	0.2	3.2	-17.8	-0.8	0.2	1.1	-1.3	-2.6	-4.0	-1.4	0.7	4.7	-0.2	19.1	-0.3	0.0
Reductase	gi|27547747	R.RHGAS.-	1/6	81625	1.3	2.1	2.9	1.7	-6.3	-3.1	0.2	0.5	1.7	1.0	1.4	0.0	0.1	0.2	-1.5	-2.1
Protein metabolism																				
Aminotransferase	gi|25797636	R.HHRGGGR.T	1/19	10076	-18.7	17.8	-0.2	-18.5	-0.5	-2.3	-15.3	-17.4	-3.2	2.0	1.0	2.9	-2.3	2.3	0.4	0.4
Leucine rich repeat containing protein kinase	gi|48716959	K.TDYESNLTVQGTQQT.-	1/6	114163	-3.4	-0.5	1.2	-1.9	-1.1	-1.4	-0.7	2.0	0.2	1.0	-1.0	-2.5	1.4	-1.6	3.0	-0.4
Proline-rich family protein	gi|18398103	R.SKHGMFGGK.R + Oxidation (M)	1/18	18383	3.2	5.8	-2.0	-4.0	1.2	5.0	-4.8	18.7	1.8	0.3	0.3	2.1	-0.4	2.3	15.8	0.6
Protease	gi|25801939	K.TPGDS.-	6/17	79816	-0.4	1.3	-17.3	-2.4	-0.7	19.1	-1.1	2.4	15.3	-14.5	-0.7	1.0	0.0	-14.7	-15.3	13.6
Ubiquitin protein	CA759059	R.DSIGKKK.K	8/27	8815	-0.3	0.4	-1.1	1.9	0.5	1.8	-2.3	7.0	-4.5	0.7	2.8	-1.2	5.1	-0.6	-0.5	0.6
Secondary metabolite metabolism																				
Flavonol 4'-sulfotransferase	gi|27547220	-.KPCMXKR.X + Oxidation (M)	1/9	67582	0.1	1.5	1.8	0.2	-1.5	-0.7	-0.1	-0.9	-0.7	1.1	1.7	-0.9	0.9	-0.8	-0.2	-1.0
NAD(P)H dependent 6'-deoxychalcone synthase	gi|27921023	K.MLYFLF.- + Oxidation (M)	1/4	79838	1.0	2.2	2.0	1.1	-0.7	-0.6	0.3	0.2	1.5	1.0	-0.7	0.5	1.6	-0.6	1.7	0.8
Os09g0368200	gi|255678841	R.YRLFMTLK.L	1/1	62278	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	HJ								KDML105							
					0	1	3	6	12	24	48	72	0	1	3	6	12	24	48	72
Sugar metabolism																				
Alcohol dehydrogenase 1	gi|20165	K.GSTVAIFGLGAVGLAAAEGAR.I	2/98	41567	2.4	-0.9	-3.8	1.3	1.3	-3.9	-5.8	-2.1	1.3	-0.9	0.8	-0.1	-0.4	-1.7	0.2	-1.8
Aldolase C-1	gi|786178	K.VSPQLIAEYTVR.A	7/137	39141	0.0	-14.9	19.1	17.4	19.4	-15.9	0.0	-17.5	-17.7	0.3	18.8	-0.4	16.3	0.0	0.0	-15.7
Beta-glucosidase					-0.5	-0.1	-0.4	-0.6	-0.5	0.2	-0.1	-0.4	0.2	0.0	-0.2	-0.3	-0.1	-0.1	0.1	0.3
Glyceraldehyde-3-phosphate dehydrogenase	gi|19880027	R.IAWEEPFGPVLPVIR.I	3/73	54856	-0.7	-0.3	0.7	-0.8	1.3	1.4	-1.7	-0.3	-0.9	1.5	1.6	0.5	-0.6	0.5	-0.5	0.5
Glycogenin glucosyltransferase	gi|8335675	-.PTKGL.-	1/9	9078	-4.1	0.0	20.0	-18.2	0.0	0.0	12.9	0.0	0.0	0.0	0.0	0.0	16.0	5.1	0.0	16.6
Glycosyl transferase ypfP	gi|27547464	K.GSASPWQAAAGACGCPSRCR.T	1/5	29011	-14.9	0.8	0.1	16.7	0.4	19.4	-4.7	0.3	0.2	-0.7	-0.6	-1.0	0.1	0.2	18.5	-15.7
Histidinol dehydrogenase	CA763212	-.SFFQA.-	1/10	26061	-4.6	0.3	-5.0	-3.2	1.9	0.6	-0.5	-0.8	-2.3	-0.3	2.6	-0.2	-2.9	3.2	0.7	1.2
Sucrose synthase	gi|20374	K.SIGNGVQFLNR.H	7/60	92568	-18.8	22.0	-0.8	1.0	-1.7	-2.3	2.6	-13.9	-0.6	-1.7	0.5	-2.1	-0.8	0.6	1.0	-1.4
Signal transduction																				
Ankyrin-like protein	BU099192	R.ELGPGL.-	1/16	12208	2.3	0.0	-15.4	15.7	0.5	-16.8	17.3	16.7	-3.0	-6.1	0.0	-5.5	4.0	-0.6	-17.8	0.2
Serine/threonine-specific protein kinase	gi|50251369	K.KEVDGESSKR.R	3/22	84451	1.0	1.5	2.5	2.4	0.0	-3.2	-16.2	-2.4	-0.2	-4.9	7.6	-4.3	-4.1	-0.9	2.1	-0.6
OSK3	gi|25806829	R.FFPAIMNK.G	1/1	22090	0.0	0.0	0.0	-16.1	-0.7	0.6	-1.9	0.0	-2.1	-15.4	2.6	18.6	1.5	0.5	0.0	-1.5

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	HJ								KDML105							
					0	1	3	6	12	24	48	72	0	1	3	6	12	24	48	72
Transcription																				
CAA303717.1 protein	gi|5777629	R.RSSDLLNAADDDQGFAADA.-	2/56	37205	-2.5	-1.1	4.8	-1.4	-3.1	1.1	-0.5	-5.5	1.8	-1.3	-1.1	0.5	-1.1	-19.9	-1.5	0.4
dbp-5 protein	gi|8334862	R.MLQLGGPR.Q + Oxidation (M)	1/11	35216	-19.9	0.0	0.0	-17.1	0.0	-0.9	0.0	-16.5	3.4	-3.4	2.0	-4.7	1.9	0.7	-0.4	4.1
Em binding protein-1a	CA757353	-.LETAH.-	1/8	22861	0.1	-16.0	-14.2	-15.9	-16.1	-17.1	0.0	0.0	0.0	-17.8	0.0	-0.8	-0.9	0.6	1.7	-1.6
F-box domain containing protein	gi|77556383	.GLGLCGCAINHGVISR.A	1/1	67301	-3.4	-0.5	1.2	-1.9	-1.1	-1.4	-0.7	2.0	0.2	1.0	-1.0	-2.5	1.4	-1.6	3.0	-0.4
F2K11.18	gi|25803017	-.PSAQT.-	1/9	25432	-1.2	-16.9	0.0	0.3	0.8	0.0	-6.7	-11.9	0.0	0.0	-0.6	0.0	2.0	-15.9	0.0	-15.8
Gibberellin-stimulated transcript 1 like protein	gi|25801810	R.TAPTARSR.R	1/22	54417	2.4	1.7	-5.1	1.6	2.9	0.4	1.2	2.3	0.8	0.7	2.4	-3.9	-1.7	0.6	3.1	2.3
HARP protein	gi|25803016	K.TLRNVRT.-	1/1	67612	2.4	1.7	-5.1	1.6	2.9	0.4	1.2	2.3	0.8	0.7	2.4	-3.9	-1.7	0.6	3.1	2.3
HD-Zip protein	gi|25802926	R.YAFTKVVFI.-	1/3	78151	-2.8	0.8	-1.2	-1.9	-0.7	0.1	-1.0	-1.5	1.8	-1.1	0.2	-1.8	3.0	-0.1	0.4	0.1
Homeodomain leucine zipper protein	gi|25799785	-.LRPNII.-	1/9	22392	-19.9	-4.5	0.5	-15.3	15.7	-0.6	-14.3	-15.3	1.3	-3.7	-2.7	-3.4	1.6	1.3	-0.4	-0.7
Integrase core domain	gi|62733246	K.AYIEKVLMK.F + Oxidation (M)	1/6	115339	0.0	0.0	0.0	0.0	0.0	0.0	1.2	17.3	17.2	0.0	1.5	-17.6	0.0	-0.9	0.0	0.0
Lysophospholipase homolog	CA760360	-.FCMAQLSQ.- + Oxidation (M)	1/7	26565	0.0	0.0	0.0	0.0	0.0	0.0	-17.4	0.0	-20.7	1.0	-4.7	-16.8	0.0	16.3	0.0	0.0
Opa-interacting protein	gi|25806125	R.KEEASDY.-	1/9	25333	17.2	5.4	14.8	-2.1	-2.4	18.4	12.8	0.0	0.2	-0.4	15.4	-1.0	-0.1	-1.4	0.4	-16.0
Origin recognition complex subunit 2	CA759483	-.RSESPLMAK.I + Oxidation (M)	1/8	25328	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-0.6	0.0	0.0	-16.3	-16.3	-17.0	0.0	-18.5

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	HJ								KDML105							
					0	1	3	6	12	24	48	72	0	1	3	6	12	24	48	72
PCNA	CA756267	-.LCMNSLYR.E + Oxidation (M)	1/17	24159	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-20.6	0.0	0.0	0.0	0.0	0.0	0.0
Phytepsin precursor	CA756102	R.TXSESGRSTQSRSSTWPTAPR.G	2/6	23116	5.4	0.9	-2.7	-2.5	0.2	1.3	0.9	-17.9	-5.7	1.2	-1.7	2.2	-3.6	0.0	5.0	-0.2
P0432B10.4	gi|20161462	R.FAVKALMKR.R	1/7	49353	-14.6	0.0	0.0	0.0	-17.7	19.6	17.3	0.0	14.6	16.7	0.0	16.3	16.1	0.0	0.0	0.0
psi-h precursor	BE040299	-.LSSNNGASS.-	1/5	22334	1.5	1.8	-0.7	0.9	0.9	0.2	-1.6	-0.5	-3.6	-0.5	-0.7	0.2	0.2	1.8	-0.9	-0.2
RNA binding protein	gi|8335896	-.LEGVVGLGL.-	1/7	80312	0.3	0.1	-6.6	-0.6	1.9	3.0	-6.5	-3.1	0.8	-3.0	3.4	-2.4	-0.4	2.3	0.7	-3.5
Slbp-P1	CA762496	R.EGTATFA.-	1/10	25778	0.0	0.0	0.0	0.0	0.0	-15.6	0.0	15.0	0.0	0.0	0.0	0.0	14.9	0.0	16.9	0.0
Transcription factor	gi|25803301	K.ALGSH.-	1/10	62034	0.7	-0.3	2.5	-0.8	1.2	0.3	-0.6	-1.1	1.7	0.1	0.2	0.2	-18.0	14.2	-0.5	0.9
Unclassified retrotransposon protein	gi|242117496	K.WSLARNFK.A	4/42	145798	-17.9	0.0	0.0	0.0	0.0	-18.5	-13.1	-15.8	3.2	-3.5	-2.8	3.4	0.9	3.9	1.0	-1.9
zinc finger, DHHC domain containing 5	CA753613	-.IGYSGGLK.S	1/10	8857	0.6	-1.0	-1.6	0.6	-3.7	-0.8	-1.6	-1.2	-3.2	0.3	0.2	-1.4	-3.0	-1.9	0.9	0.2
Translation																				
Aspartyl protease	gi|25797766	K.KKNRMK.Q	1/17	22163	-19.3	19.0	-0.6	0.7	-1.9	-0.6	-15.4	-18.0	-3.1	-1.1	-0.6	-1.4	-2.9	6.0	2.3	-3.8
Cytochrome c oxidase subunit III	CA762510	K.AQKNEMK.K	2/6	25411	-0.4	-2.0	0.4	15.4	0.0	-1.7	17.7	-17.2	2.3	0.7	-0.4	0.9	-1.7	0.9	-0.5	1.6
Elongation factor ef-2	BE040066	R.HMRAG.- + Oxidation (M)	1/2	35389	0.0	0.0	0.0	0.7	-15.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.1	-16.6	16.7	-16.1
40S ribosomal protein S15	CA758185	R.ATAFPNP.-	1/7	32243	0.7	2.6	-0.2	-1.9	-2.6	1.1	-0.5	-2.4	-19.1	18.6	-2.1	0.5	1.4	-0.4	2.0	1.2

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	HJ								KDML105							
					0	1	3	6	12	24	48	72	0	1	3	6	12	24	48	72
60s ribosomal protein l27	BE040610	R.VLADHLLR.V	2/11	33450	0.4	-1.0	0.8	0.6	0.2	1.0	-1.9	-18.0	-0.2	1.5	-0.1	-2.2	-3.9	-2.5	4.6	0.7
60s ribosomal protein l6	gi|8334650	R.RPGSH.-	1/5	21429	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	-2.7	0.4	3.2	3.9	-17.1	1.0	-14.9
60S ribosomal protein L24	CA756401	R.LNVDIFD.-	1/4	23148	2.5	0.0	-1.1	-1.5	-0.2	-0.1	1.3	0.2	0.0	19.7	18.2	-0.3	1.0	-0.9	-1.1	-1.6
Translation elongation factor EF-1 alpha	CA753016	K.RGLEX.-	1/6	25917	2.3	0.0	-15.4	15.7	0.5	-16.8	17.3	16.7	-3.0	-6.1	0.0	-5.5	4.0	-0.6	-17.8	0.2
Translation initiation factor eIF-6	CA763564	R.SPPCLGTGH.-	1/3	26761	-1.8	17.8	-15.8	2.9	-19.0	-20.0	-2.7	-0.2	0.0	0.0	17.9	1.1	0.0	-0.5	0.6	0.0
von hippel-lindau binding protein 1	BE040150	K.IVPSSGRS.-	1/4	34671	16.3	-0.3	0.0	-18.6	2.1	0.0	-0.4	17.1	17.0	0.0	-0.6	0.1	-20.8	0.0	16.5	1.0
Cell defense and rescue system																				
ABC transporter ATP-binding protein	gi|25806938	-.ASLSTFR.G	1/12	64258	-0.8	0.4	0.1	0.3	0.8	-1.1	-2.1	-1.0	-0.6	0.3	0.2	-2.4	2.5	-1.5	1.0	-0.2
Aquaporin	gi|8334728	-.IKGCIM.-	1/1	100816	2.0	1.1	0.1	-0.8	-1.5	0.0	-1.9	-0.8	-0.4	1.1	1.3	-0.1	2.0	0.9	-0.1	0.8
Basidic peroxidase					1.6	5.4	4.5	4.0	10.8	3.5	15.4	23.9	-11.9	2.5	5.2	-2.0	5.5	8.6	-13.9	-13.4
Cyclophilin	gi|8335257	R.SFPVLPL.-	1/6	76662	-4.1	1.3	2.5	7.6	0.6	0.5	0.0	-0.2	3.0	-3.0	-1.1	4.7	-1.2	3.3	-2.9	-0.5
Cytochrome P450 71D8	CA765579	R.CSSRNGFTVK.R	1/15	29857	-0.8	-0.6	-0.6	1.6	1.7	-0.3	-3.2	-16.1	-3.7	0.4	2.5	2.3	2.4	-1.5	2.7	-1.2
Enolase	gi|780372	K.VNQIGSVTESIEAVK.M	4/54	48299	0.0	1.5	-0.6	1.6	0.1	1.6	-1.4	-0.9	-0.2	-0.2	1.3	0.2	0.3	-19.7	-2.0	0.2

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	HJ								KDML105							
					0	1	3	6	12	24	48	72	0	1	3	6	12	24	48	72
Glutathione s-transferase	gi|25798958	K.KKSTAGGR.S	2/12	22473	-17.3	-2.9	0.3	3.0	19.2	-4.8	1.5	-20.3	0.5	-0.6	1.6	0.0	-2.4	3.0	1.2	1.2
HUMMDR1 Human P-glycoprotein	gi|2796454	K.GGPXL.-	1/2	5621	12.0	0.0	-15.0	0.5	0.0	0.0	12.8	14.7	-16.8	0.0	-0.3	13.4	18.4	2.0	-16.6	-2.8
Hypersensitivity-induced response-like protein	gi|25803492	K.NSMLLAK.Q + Oxidation (M)	1/9	25374	18.4	2.9	0.0	0.0	-19.4	0.0	-6.2	-3.2	0.0	0.0	15.6	-16.5	0.0	1.3	0.0	0.0
Iron receptor	gi|25798451	K.GARAGTVAR.R	1/6	18003	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	17.0	0.0	2.2	2.6	2.0	1.3	-0.8
Manganese superoxide dismutase	gi|601869	K.NVRPDYLSNIWK.V	1/31	24932	-1.3	0.9	2.7	-1.3	4.2	2.8	-1.3	-2.4	-1.8	-1.1	-1.3	0.9	2.1	-1.2	-2.3	0.8
Metallothionein-like protein	gi|2800520	R.GGXXP.-	1/2	47125	0.3	2.6	-16.2	-0.1	-0.3	0.4	-0.1	0.3	-1.2	7.7	-2.7	0.1	-0.7	14.8	-14.3	-0.4
Neutral peroxidase					17.6	4.2	4.8	-1.4	16.6	7.8	12.8	31.3	-16.3	3.2	5.6	-5.2	7.6	10.8	-21.3	-20.4
OJ000114_01.12	gi|39545657	R.GSGNGFEGLK.T	1/22	40790	0.0	0.0	0.0	0.0	0.0	0.0	18.1	0.0	-16.2	0.0	15.5	15.9	14.8	0.0	0.0	0.0
Pathogenesis-related protein PR-10b	CA759435	R.RAXRLGVGGAGVEGLSDAPAMPK.V	1/11	24168	0.3	2.5	0.8	-0.7	-0.6	0.1	-0.5	-1.3	-0.2	1.6	-0.1	0.7	0.0	1.0	-0.4	4.4
Phenylalanine ammonia-lyase	gi|8336796	K.HLVALLAGELK.L	1/13	69395	-19.4	-0.4	4.4	15.7	0.0	1.2	14.0	-18.3	5.5	1.2	3.4	-0.5	1.1	-0.3	-1.7	-2.8
Phosphatidylinositol-4-phosphate-5-kinase	gi|25803548	R.YTSIQR.M	1/21	69009	-0.3	5.9	1.9	0.0	0.3	-2.9	-3.3	-0.3	1.3	-14.5	-2.1	-2.1	-9.1	17.0	-0.1	-2.6

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	HJ								KDML105							
					0	1	3	6	12	24	48	72	0	1	3	6	12	24	48	72
Plasma membrane ATPase					0.0	0.0	0.0	-0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Retroelement	gi|19881553	R.GKAGELGVGAPR.D	1/2	92534	0.0	0.0	0.0	0.0	0.0	0.0	1.2	17.3	17.2	0.0	1.5	-17.6	0.0	-0.9	0.0	0.0
Salivary proline-rich glycoprotein precursor	gi|8334645	R.ADKAKQAG.-	1/9	104377	-1.5	-0.4	-0.6	5.5	-0.8	-0.6	1.0	-2.7	1.0	0.8	3.2	-2.4	-0.6	0.8	-1.6	-1.3
Total ATPase					-0.1	0.0	0.0	0.0	0.0	0.0	-0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0
Transporting																				
Brefeldin A-sensitive Golgi protein - like	CA762643	K.SSLMSFKK.K	1/11	20874	1.0	0.5	1.4	1.1	-1.1	-2.5	0.1	-2.5	17.5	0.6	-3.1	0.2	2.8	-0.6	0.5	-0.2
GTP-binding protein	CA757475	K.MQIQLH.- + Oxidation (M)	1/17	23646	-0.5	1.9	-0.7	-0.7	0.6	-0.6	0.3	-1.7	-0.3	-1.8	-1.1	1.1	0.0	-2.3	1.0	-0.5
Multispanning membrane protein	BE040128	-.SSIDLNF.-	1/6	37181	0.6	-1.0	-1.6	0.6	-3.7	-0.8	-1.6	-1.2	-3.2	0.3	0.2	-1.4	-3.0	-1.9	0.9	0.2
SEC13-related protein	CA754964	-.YRFTIS.-	1/16	23420	-0.5	1.9	-0.7	-0.7	0.6	-0.6	0.3	-1.7	-0.3	-1.8	-1.1	1.1	0.0	-2.3	1.0	-0.5
Secretory carrier membrane protein	gi|25803448	K.GFGGIHK.G	1/5	69328	0.6	-3.7	-0.3	-19.0	-20.7	0.2	-2.2	-2.0	-1.9	-18.1	2.5	0.9	0.0	-2.4	1.2	2.7
p125 protein	gi|25799240	R.CPTGSPR.R	1/7	23044	-18.7	17.8	-0.2	-18.5	-0.5	-2.3	-15.3	-17.4	-3.2	2.0	1.0	2.9	-2.3	2.3	0.4	0.4
Synaptobrevin-like protein	BE040833	R.ADAGGGGGGR.R	1/27	30595	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0	17.3
Storage protein																				
Preproglutelin	AA752620	-.MXCCSSLY.- + Oxidation (M)	1/18	16976	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-21.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	HJ								KDML105							
					0	1	3	6	12	24	48	72	0	1	3	6	12	24	48	72
Prolamin PPROL 14 precursor	CA762870	-.ADPRGIVPTVPPFSR.K	1/13	24031	-2.0	2.4	-0.4	-1.2	0.8	-1.3	0.1	-0.4	1.6	1.6	0.1	0.4	-0.9	-1.1	0.2	1.2
DNA processing																				
mutM homologue-1	gi|25806579	R.QGKMVVAR.R	1/11	26107	-16.1	0.7	0.2	-2.6	17.3	-0.3	1.0	-3.3	0.4	-1.3	-0.4	1.7	1.4	2.1	1.8	-0.9
Nucleoside diphosphate kinase	AA752790	K.NGXPKIK.N	1/10	26566	-0.5	-0.3	-1.1	2.7	0.7	-0.3	-1.3	-1.7	-2.1	2.5	-0.6	-1.7	-2.8	-4.2	2.2	-3.1
Ribonucleosie reductase 1 (Large chain)	gi|25806543	-.LQGLP.-	1/6	26009	2.7	4.4	0.0	1.1	-1.6	3.4	-3.1	-0.6	15.5	15.3	-15.2	14.4	-0.8	2.8	-1.0	2.0
Unknown																				
agCP12256	CA757055	-.VWELIQILKIPKE.-	1/12	20801	-2.0	2.4	-0.4	-1.2	0.8	-1.3	0.1	-0.4	1.6	1.6	0.1	0.4	-0.9	-1.1	0.2	1.2
agCP5654	gi|25803664	R.LFENKY.-	1/12	26289	-1.0	-2.8	4.3	-0.6	2.5	1.3	0.0	-1.6	-1.2	-1.8	0.4	-1.6	0.9	0.0	0.9	-0.9
agCP8018	CA759626	R.AVVLSR.Q	2/31	26861	0.7	-0.4	0.2	-0.2	-0.2	1.2	0.1	-0.2	0.4	-0.7	2.0	-2.4	0.1	2.5	0.8	0.1
mtn12	BE040684	-.RLILHPPH.-	1/4	39754	-0.9	1.5	0.1	-1.1	0.2	-1.4	-2.5	-2.5	-0.1	0.8	-2.8	1.6	0.2	-0.4	-1.2	-1.2
OSJNBa0014K14.4	gi|70663918	M.APPLLPRGAALLLLLR.S	1/1	68298	-3.4	-0.5	1.2	-1.9	-1.1	-1.4	-0.7	2.0	0.2	1.0	-1.0	-2.5	1.4	-1.6	3.0	-0.4
OSJNBa0029C04.3	gi|25797412	R.DTESR.A	1/5	83946	1.1	1.1	0.9	-0.1	-0.3	1.5	1.9	-0.6	-2.4	0.4	0.8	-0.4	-0.3	0.4	-2.2	1.4
OSJNBa0038O10.8	gi|38344436	R.RSSPGLVAAPR.L	2/11	56352	0.0	0.0	0.0	0.0	0.0	0.0	1.2	17.3	17.2	0.0	1.5	-17.6	0.0	-0.9	0.0	0.0

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	PT1 0	1	3	6	12	24	48	72
Skeleton and cell organization												
Extensin-like protein	CA753366	R.GQRGYSXXRS.-	1/17	29486	10.2	0.2	0.3	1.0	0.0	-7.3	-17.1	0.0
Myosin heavy chain	gi|31193918	R.LMINNR.I + Oxidation (M)	25/30	170023	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Protein ABIL1	gi|255541748	K.AGPVS.-	1/27	34537	-0.6	-1.6	-15.0	2.7	0.4	-4.1	-0.8	0.0
Zeatin O-glucosyltransferase	CA764857	R.TLTLGQGSR.S	1/12	25912	2.2	2.0	0.5	-1.4	0.4	-0.3	-3.1	-1.7
Energy metabolism												
Aacyl-coa synthetase	BE041069	R.GPTSSGGSR.H	1/11	25215	1.0	1.8	1.5	-1.0	0.9	0.1	0.7	-2.7
beta-hydroxyacyl-acp dehydratase	gi|8334683	-.LQGIP.-	1/6	36394	-0.3	-17.1	-4.6	15.3	13.8	-5.3	0.1	-17.8
NADH dehydrogenase subunit 5	CA756211	R.ELNGN.-	1/3	21618	-0.1	-16.2	-0.7	14.4	-0.6	0.1	-0.6	0.0
Phosphoribosylamidoimidazole-succinocarboxamide synthase precursor	CA764505	R.KCYIXG.-	2/16	28122	-0.8	-0.9	2.2	-1.2	-0.4	1.0	-1.0	-3.2

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	PT1 0	1	3	6	12	24	48	72
3-oxoacyl-[acyl-carrier-protein] synthase i	gi|8335079	-.PASFV.-	1/5	94375	4.8	-3.1	-4.5	3.3	0.2	0.1	-0.5	-4.3
Reductase	gi|27547747	R.RHGAS.-	1/6	81625	3.4	1.2	0.7	-1.2	1.6	2.5	-1.5	-2.8
Protein metabolism												
Aminotransferase	gi|25797636	R.HHRGGGR.T	1/19	10076	-1.8	16.9	-1.7	3.8	0.5	-17.1	2.3	-0.3
Leucine rich repeat containing protein kinase	gi|48716959	K.TDYESNLTVQGT QQT.-	1/6	114163	-0.6	-0.9	-1.9	0.2	0.9	-1.0	2.6	-2.0
Proline-rich family protein	gi|18398103	R.SKHGMFGGK.R + Oxidation (M)	1/18	18383	-0.1	-0.4	0.5	-1.6	0.6	0.0	-1.7	-0.5
Protease	gi|25801939	K.TPGDS.-	6/17	79816	1.8	-2.1	1.1	-0.8	0.7	0.7	1.4	-3.7
Ubiquitin protein	CA759059	R.DSIGKKK.K	8/27	8815	1.0	1.5	-0.2	-0.4	0.4	1.9	-1.7	-17.4
Secondary metabolite metabolism												
Flavonol 4'-sulfotransferase	gi|27547220	-.KPCMXKR.X + Oxidation (M)	1/9	67582	2.0	-0.7	-0.9	0.9	-0.9	3.9	2.1	-2.0
NAD(P)H dependent 6'-deoxychalcone synthase	gi|27921023	K.MLYFLF.- + Oxidation (M)	1/4	79838	3.9	-0.7	-0.2	0.7	1.6	2.1	1.9	-0.5
Os09g0368200	gi|255678841	R.YRLFMTLK.L	1/1	62278	1.6	15.5	15.0	0.3	-1.1	-14.1	0.5	0.1

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	PT1 0	1	3	6	12	24	48	72
Sugar metabolism												
Alcohol dehydrogenase 1	gi|20165	K.GSTVAIFGLGAVGLAAAEGAR.I	2/98	41567	-2.9	2.6	2.9	-2.7	0.5	-0.9	0.0	0.6
Aldolase C-1	gi|786178	K.VSPQLIAEYTVR.A	7/137	39141	18.6	-15.4	-16.5	0.0	0.0	0.0	-1.4	0.0
Beta-glucosidase					0.2	0.0	-0.1	0.7	0.1	-0.1	-0.5	0.3
Glyceraldehyde-3-phosphate dehydrogenase	gi|19880027	R.IAWEEPFGPVLPVIR.I	3/73	54856	-2.1	-3.3	-0.9	2.9	-0.6	-0.2	-0.1	-0.7
Glycogenin glucosyltransferase	gi|8335675	-.PTKGL.-	1/9	9078	0.0	0.0	0.0	0.0	15.7	-2.3	-14.7	0.0
Glycosyl transferase ypfP	gi|27547464	K.GSASPWQAAAGACGCPSRCR.T	1/5	29011	0.7	-16.1	15.7	0.9	15.7	15.8	0.3	-16.1
Histidinol dehydrogenase	CA763212	-.SFFQA.-	1/10	26061	0.1	3.9	-0.1	-1.5	-1.3	0.4	-1.9	-1.2
Sucrose synthase	gi|20374	K.SIGNGVQFLNR.H	7/60	92568	1.8	-3.1	-0.7	4.0	0.3	-15.9	2.3	-1.3
Signal transduction												
Ankyrin-like protein	BU099192	R.ELGPGL.-	1/16	12208	1.5	2.5	0.3	-0.3	-18.2	0.3	0.2	-2.6
Serine/threonine-specific protein kinase	gi|50251369	K.KEVDGESSKR.R	3/22	84451	3.1	-0.9	1.3	-0.4	0.1	-1.3	-0.4	-2.9
OSK3	gi|25806829	R.FFPAIMNK.G	1/1	22090	-19.4	-18.2	-13.8	0.5	0.0	16.5	16.1	-17.1

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	PT1 0	1	3	6	12	24	48	72
Transcription												
CAA303717.1 protein	gi|5777629	R.RSSDLLNAADDDQGFAADA.-	2/56	37205	-0.3	2.0	-0.2	0.4	-3.0	4.5	-1.8	-0.7
dbp-5 protein	gi|8334862	R.MLQLGGPR.Q + Oxidation (M)	1/11	35216	16.9	17.5	15.1	-0.1	0.3	-15.0	-14.5	4.4
Em binding protein-1a	CA757353	-.LETAH.-	1/8	22861	16.3	0.0	0.0	0.0	15.4	-5.3	-18.5	0.0
F-box domain containing protein	gi|77556383	.GLGLCGCAINHGVISR.A	1/1	67301	-0.6	-0.9	-1.9	0.2	0.9	-1.0	2.6	-2.0
F2K11.18	gi|25803017	-.PSAQT.-	1/9	25432	-18.0	-19.6	-17.9	0.0	0.0	-16.3	0.0	14.9
Gibberellin-stimulated transcript 1 like protein	gi|25801810	R.TAPTARSR.R	1/22	54417	-1.2	1.6	-1.1	0.3	0.2	-3.7	3.7	-1.9
HARP protein	gi|25803016	K.TLRNVRT.-	1/1	67612	-1.2	1.6	-1.1	0.3	0.2	-3.7	3.7	-1.9
HD-Zip protein	gi|25802926	R.YAFTKVVFI.-	1/3	78151	3.8	-1.6	1.7	2.3	1.1	2.6	-0.4	-0.1
Homeodomain leucine zipper protein	gi|25799785	-.LRPNII.-	1/9	22392	18.4	15.4	-2.7	-0.2	-2.3	-0.9	-0.2	1.6
Integrase core domain	gi|62733246	K.AYIEKVLMK.F + Oxidation (M)	1/6	115339	15.1	0.4	1.0	-1.4	-15.2	0.4	-16.4	-19.3
Lysophospholipase homolog	CA760360	-.FCMAQLSQ.- + Oxidation (M)	1/7	26565	15.7	16.8	14.8	0.0	0.0	0.0	0.0	0.0
Opa-interacting protein	gi|25806125	R.KEEASDY.-	1/9	25333	-17.2	0.0	0.0	-16.8	-16.7	15.9	-0.8	0.0
Origin recognition complex subunit 2	CA759483	-.RSESPLMAK.I + Oxidation (M)	1/8	25328	0.0	0.0	0.0	18.0	15.2	0.0	0.0	0.0

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	PT1 0	1	3	6	12	24	48	72
PCNA	CA756267	-.LCMNSLYR.E + Oxidation (M)	1/17	24159	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Phytepsin precursor	CA756102	R.TXSESGRSTQSRSS TWPTAPR.G	2/6	23116	-0.2	2.0	-0.7	-1.3	-1.8	0.8	-0.6	-14.6
P0432B10.4	gi|20161462	R.FAVKALMKR.R	1/7	49353	15.7	-17.2	-1.3	0.0	-17.1	15.0	-0.5	15.0
psi-h precursor	BE040299	-.LSSNNGASS.-	1/5	22334	0.5	-1.5	0.8	-0.7	1.6	3.5	1.6	-1.4
RNA binding protein	gi|8335896	-.LEGVVGLGL.-	1/7	80312	-1.1	-1.3	-2.8	-0.7	5.8	2.2	-3.0	-2.3
Slbp-P1	CA762496	R.EGTATFA.-	1/10	25778	0.0	0.0	15.6	0.0	0.0	-13.2	0.0	0.0
Transcription factor	gi|25803301	K.ALGSH.-	1/10	62034	1.0	-2.2	-0.4	-1.3	-0.3	-1.5	-1.2	-1.6
Unclassified retrotransposon protein	gi|242117496	K.WSLARNFK.A	4/42	145798	-16.2	15.6	-1.2	0.4	-0.9	-14.4	3.1	-2.0
zinc finger, DHHC domain containing 5	CA753613	-.IGYSGGLK.S	1/10	8857	1.4	0.0	1.1	0.3	1.2	-0.1	-1.0	-0.8
Translation												
Aspartyl protease	gi|25797766	K.KKNRMK.Q	1/17	22163	1.9	-1.2	2.1	-2.0	-3.1	-15.8	2.3	0.7
Cytochrome c oxidase subunit III	CA762510	K.AQKNEMK.K	2/6	25411	2.7	1.3	-0.8	-0.7	3.8	2.5	-16.1	-17.0
Elongation factor ef-2	BE040066	R.HMRAG.- + Oxidation (M)	1/2	35389	16.4	16.2	0.0	14.6	-15.3	0.0	15.6	-17.0
40S ribosomal protein S15	CA758185	R.ATAFPNP.-	1/7	32243	1.3	0.7	0.3	0.5	1.4	0.5	-1.5	-3.2

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	PT1 0	1	3	6	12	24	48	72
60s ribosomal protein l27	BE040610	R.VLADHLLR.V	2/11	33450	2.8	0.1	0.9	1.8	0.6	-0.4	-0.7	-0.1
60s ribosomal protein l6	gi|8334650	R.RPGSH.-	1/5	21429	0.0	14.8	4.4	0.0	0.0	-15.5	-12.7	1.7
60S ribosomal protein L24	CA756401	R.LNVDIFD.-	1/4	23148	1.0	-1.5	2.9	0.0	1.3	-4.3	14.9	-16.9
Translation elongation factor EF-1 alpha	CA753016	K.RGLEX.-	1/6	25917	1.5	2.5	0.3	-0.3	-18.2	0.3	0.2	-2.6
Translation initiation factor eIF-6	CA763564	R.SPPCLGTGH.-	1/3	26761	2.4	-17.2	17.6	-16.2	0.0	1.3	-17.0	-1.1
von hippel-lindau binding protein 1	BE040150	K.IVPSSGRS.-	1/4	34671	1.4	0.0	19.0	0.0	-1.3	2.1	0.0	16.8
Cell defense and rescue system												
ABC transporter ATP-binding protein	gi|25806938	-.ASLSTFR.G	1/12	64258	3.6	-0.7	-1.3	0.6	0.6	1.4	-0.7	-0.5
Aquaporin	gi|8334728	-.IKGCIM.-	1/1	100816	0.7	0.4	-1.1	0.3	2.2	0.9	-2.4	-1.9
Basidic peroxidase					-4.3	19.7	21.6	8.1	-2.5	27.1	17.1	22.3
Cyclophilin	gi|8335257	R.SFPVLPL.-	1/6	76662	-0.8	0.5	-1.1	0.3	-0.4	-1.9	0.9	-0.6
Cytochrome P450 71D8	CA765579	R.CSSRNGFTVK.R	1/15	29857	1.6	-1.3	-2.1	0.1	3.2	-1.8	-5.1	-1.3
Enolase	gi|780372	K.VNQIGSVTESIEAVK.M	4/54	48299	-0.3	0.7	-0.9	2.5	-2.3	2.5	1.8	-0.8

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	PT1							
					0	1	3	6	12	24	48	72
Glutathione s-transferase	gi|25798958	K.KKSTAGGR.S	2/12	22473	14.9	-16.8	0.7	1.1	4.0	-16.2	3.4	-17.8
HUMMDR1 Human P-glycoprotein	gi|2796454	K.GGPXL.-	1/2	5621	0.0	-0.1	-1.7	16.2	16.2	0.1	0.5	3.2
Hypersensitivity-induced response-like protein	gi|25803492	K.NSMLLAK.Q + Oxidation (M)	1/9	25374	0.0	-0.3	18.3	-16.1	-19.2	0.0	14.5	0.0
Iron receptor	gi|25798451	K.GARAGTVAR.R	1/6	18003	0.0	15.1	0.0	0.0	-17.3	-16.3	0.0	1.8
Manganese superoxide dismutase	gi|601869	K.NVRPDYLSNIWK.V	1/31	24932	0.7	-0.1	-0.9	0.5	4.3	1.0	-2.3	-3.9
Metallothionein-like protein	gi|2800520	R.GGXXP.-	1/2	47125	2.6	-0.2	0.8	-1.4	-0.1	3.4	2.1	3.2
Neutral peroxidase					-3.1	17.6	16.0	-3.5	-15.1	39.0	19.8	36.3
OJ000114_01.12	gi|39545657	R.GSGNGFEGLK.T	1/22	40790	-17.2	0.0	0.0	15.9	0.0	0.0	0.0	-15.7
Pathogenesis-related protein PR-10b	CA759435	R.RAXRLGVGGAGVEGLSDAPAMPK.V	1/11	24168	-2.1	-3.4	0.2	-0.8	5.8	0.5	-1.3	-0.9
Phenylalanine ammonia-lyase	gi|8336796	K.HLVALLAGELK.L	1/13	69395	2.1	17.9	0.0	0.0	1.3	0.0	0.0	17.4
Phosphatidylinositol-4-phosphate-5-kinase	gi|25803548	R.YTSIQR.M	1/21	69009	0.4	-0.4	3.0	-0.7	0.3	-0.3	0.9	1.1

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	PT1							
					0	1	3	6	12	24	48	72
Plasma membrane ATPase					-0.1	0.0	0.0	0.0	0.2	0.0	0.0	-0.1
Retroelement	gi|19881553	R.GKAGELGVGAPR.D	1/2	92534	15.1	0.4	1.0	-1.4	-15.2	0.4	-16.4	-19.3
Salivary proline-rich glycoprotein precursor	gi|8334645	R.ADKAKQAG.-	1/9	104377	0.7	1.7	-2.0	-0.2	1.2	1.9	1.8	-1.2
Total ATPase					0.1	0.0	-0.2	0.0	0.0	0.0	0.0	0.0
Transporting												
Brefeldin A-sensitive Golgi protein - like	CA762643	K.SSLMSFKK.K	1/11	20874	0.2	-0.8	-0.8	-0.9	1.7	-2.1	-2.2	-0.8
GTP-binding protein	CA757475	K.MQIQLH.- + Oxidation (M)	1/17	23646	-0.8	-0.9	2.2	-1.2	-0.4	1.0	-1.0	-3.2
Multispanning membrane protein	BE040128	-.SSIDLNF.-	1/6	37181	1.4	0.0	1.1	0.3	1.2	-0.1	-1.0	-0.8
SEC13-related protein	CA754964	-.YRFTIS.-	1/16	23420	-0.8	-0.9	2.2	-1.2	-0.4	1.0	-1.0	-3.2
Secretory carrier membrane protein	gi|25803448	K.GFGGIHK.G	1/5	69328	0.8	0.2	-0.9	3.3	0.6	1.1	-0.2	-0.8
p125 protein	gi|25799240	R.CPTGSPR.R	1/7	23044	-1.8	16.9	-1.7	3.8	0.5	-17.1	2.3	-0.3
Synaptobrevin-like protein	BE040833	R.ADAGGGGGGR.R	1/27	30595	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Storage protein												
Preproglutelin	AA752620	-.MXCCSSLY.- + Oxidation (M)	1/18	16976	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix Table C1 (Continued)

Protein	Accession number	Peptide	Match/ Score	Mass (Da)	PT1 0	1	3	6	12	24	48	72
Prolamin PPROL 14 precursor	CA762870	-.ADPRGIVPTVPPFSR.K	1/13	24031	1.0	1.8	1.5	-1.0	0.9	0.1	0.7	-2.7
DNA processing												
mutM homologue-1	gi|25806579	R.QGKMVVAR.R	1/11	26107	-2.3	-0.3	0.5	-1.8	1.5	-1.3	15.8	0.8
Nucleoside diphosphate kinase	AA752790	K.NGXPKIK.N	1/10	26566	3.3	-0.5	-0.9	1.5	2.6	0.7	-2.8	14.7
Ribonucleosie reductase 1 (Large chain)	gi|25806543	-.LQGLP.-	1/6	26009	-0.3	-17.1	-4.6	15.3	13.8	-5.3	0.1	-17.8
Unknown												
agCP12256	CA757055	-.VWELIQILKIPKE.-	1/12	20801	1.0	1.8	1.5	-1.0	0.9	0.1	0.7	-2.7
agCP5654	gi|25803664	R.LFENKY.-	1/12	26289	-0.6	0.2	2.2	0.4	2.0	-0.3	-0.6	1.6
agCP8018	CA759626	R.AVVLSR.Q	2/31	26861	1.3	-0.4	0.0	14.6	0.7	1.0	-2.5	-1.4
mtn12	BE040684	-.RLILHPPH.-	1/4	39754	1.0	0.9	-1.0	0.5	1.2	1.7	-0.2	0.2
OSJNBa0014K14.4	gi|70663918	M.APPLLPRGAALLLLLR.S	1/1	68298	-0.6	-0.9	-1.9	0.2	0.9	-1.0	2.6	-2.0
OSJNBa0029C04.3	gi|25797412	R.DTESR.A	1/5	83946	0.5	0.1	0.5	0.3	-0.8	0.5	-2.6	0.6
OSJNBa0038O10.8	gi|38344436	R.RSSPGLVAAPR.L	2/11	56352	15.1	0.4	1.0	-1.4	-15.2	0.4	-16.4	-19.3

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