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# Proteomic Alterations During Dormant Period of *Curcuma Longa* Rhizomes

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## Abstract

Proteins involved during storage of *Curcuma longa* have not been investigated in detail. Proteins and nucleic acids are considered to be essential for the sprouting period. As numerous applications of proteomic approaches have been reported in many areas of biology, biochemistry and biomedicine, we chose to use proteomic technology to study the protein expression of *Curcuma longa* (Khaminchun) rhizome during dormancy and sprouting. Microscale solution-phase isoelectric focusing (Zoom) was employed to enrich the low abundance proteins in the pH range of 5.4-10 and improve the separation of those proteins in the acidic range from 3-5.4. Samples were drawn at seven-day intervals from harvest until the commencement of sprouting. The proteomic patterns of the storage period (0, 14, 21, 42, and 70 days) were studied and identified by LC/MS/MS in these two pH ranges. High levels of glyceraldehyde-3-phosphate dehydrogenase, a glycolytic enzyme, were present and in glycosylated and phosphorylated forms. Sporamin, the major storage protein of the tuberous roots of sweet potato, was highly expressed in the dormant period and lower expression was detected in the sprouting period. Moreover, the enzyme for catalyzing the synthesis of catechin, leucoanthocyanidin reductase, was found for the first time in the *Curcuma longa* rhizome. These results represent the first proteomic patterns during the storage period of *Curcuma longa*. For the rhizomes of *Curcuma longa*, visible sprouting was observed within 70 days after harvest.

**Keywords:** Zingiberaceae; *Curcuma*; Rhizomes; Enrich; Dormancy; Sprouting

## Introduction

Curcuma is a perennial herb of the family Zingiberaceae. The rhizome of Curcuma, commonly called turmeric (*Curcuma longa* L.), has been used for centuries as spice and coloring agent. Turmeric is indigenous to South, Southwest and Southeast Asia. The traditional uses of turmeric in folk medicines are multiple and many of the therapeutic effects have been confirmed by contemporary scientific research. These include antioxidant, antiarthritic, antimutagenic, antitumor, antithrombotic, antivenom, antibacterial, antifungal, antiviral, nematocidal, choleretic, antihepatotoxic and hemagglutinating activities (Kumar et al., 2006; Sangvanich et al., 2007).

In plant physiology, dormancy is a period of arrested plant growth. It is a strategy exhibited by many plant species to enable their survival in climates unsuitable for growth such as winter or dry season. *Curcumas* will start going dormant (resting) in the fall (October), even in warm tropical climates and the rhizomes will grow during summer (Panneerselvam, 1998). The levels of sugars, starch and the activities of enzymes involved in carbohydrate metabolism, respiration, glycolysis, tricarboxylic acid (TCA) cycle and oxidative pentose phosphate pathway (PPP) have been studied during the early period of dormancy and sprouting (Panneerselvam et al., 2007).

At the molecular level, little is known regarding the protein changes during storage of *Curcuma longa*. Proteins and nucleic acids are considered to be essential for the sprouting of potato buds (Tuan and Bonner, 1964). As numerous applications of proteomic approaches have been reported in many areas of biology, biochemistry and biomedicine, we chose to use proteomics to study the protein expression of *Curcuma longa* (Khaminchun) rhizome during dormancy and sprouting. Microscale solution-phase isoelectric focusing (Zoom) was employed to enrich the low abundant proteins in the pH range from 5.4-10 and separate the acidic group (3-5.4) of proteins as well. The two pH ranges of proteins of different periods of dormancy of *Curcuma longa* were compared and proteins identified using LC/MS/MS.

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## Methods

### Protein Extraction

Fresh rhizomes of *Curcuma longa* were harvested from Ratchaburi province after the aerial parts have fully dried up and stored in the dark. This period is considered to be the initial period of dormancy. Mature healthy rhizomes of uniform size and weight were kept at 28±2ºC and relative humidity of 65-75% in a biophotochamber. Samples were collected at 7-day intervals from harvest until sprouting.

*Curcuma longa* rhizomes (15 grams) were peeled to remove the skin before ground in liquid nitrogen using a mortar and pestle, and suspended in 30 ml of extraction buffer (0.7 M sucrose, 0.5 M Tris-HCl, 30 mM HCl, 0.1 M KCl and 1% v/v β-mercaptoethanol). The mixture was extensively homogenized and stored at 4°C for at least 30 minutes. After centrifugation at 4,000g for 10 minutes, the supernatant was transferred and kept at 4°C. The plant tissues were resuspended in extraction buffer two times. Fifty milliliters of water-saturated phenol was added to the supernatant, mixed and kept at 4°C for 60 minutes. After centrifugation at 8,000g for 10 minutes, the upper phenol phase was removed and resuspended twice in water-saturated phenol (Hurkman and Tanaka, 1986). Proteins were precipitated from the phenol phase of 200 ml 0.1 M ammonium acetate, dissolved in methanol and stored overnight at – 20°C. The sample was centrifuged at 4,000g for 10 minutes. The pellet was dissolved in 10 ml of cold water and sonicated for 3 minutes. The proteins were precipitated in 9 volumes of cold acetone. The solution was left at -20 C for at least 4 hours and then centrifuged at 4,000g for 10 minutes. The supernatant was discarded and the pellet was air-dried briefly until the smell of acetone had disappeared. The amount of protein was determined by using Bradford method.

### Microscale Solution-phase Isoelectric Focusing (Zoom) of Protein Extract

For in-solution IEF fractionation, a microscale solution-phase isoelectric focusing (ZOOM) IEF fractionator was used (Invitrogen, Carlsbad, CA, USA). Proteins were reduced by dissolving with 50 mM DTT (USB Corporation Cleveland, OH, USA), 7.7 M urea (ICN), 2.2 M thiourea (Sigma, St Louis, MO, USA), and 4.4% CHAPS (Calbiochem, San Diego, CA, USA). The reduced proteins were then alkylated by adding 100 mM iodoacetamide for 30 min and incubated in the dark. Insoluble matter was removed by 20 min centrifugation at 12,000 rpm. To remove iodoacetamide, the protein lysate was precipitated using cold acetone. The pellet was resuspended in 7.7 M urea, 2.2 M thiourea, 4.4% CHAPS, 10 mM DTT and 0.8% ampholine at an approximate protein concentration of 0.6 mg/ml. The Zoom-IEF fractionator was assembled with disks pH 3.0, pH 5.4 and pH 10.0. One thousand and three hundred µl lysate was loaded between disk pH 3.0-5.4 and 650 µl was loaded between disk pH 5.4-10.0. The proteins were focused for 20 min at 70 V, 160 min at 70-600 V, and 120 min at 600 V. After separation each fraction was transferred into a clean microcentifuge tube.

### Two-dimensional Polyacrylamide Gel electrophoresis (2-D PAGE)

2-D PAGE was performed using the immobiline/polyacrylamide system. Samples were applied by overnight in-gel rehydration of 70 mm ( analytical runs ). pH 4-7 IPG gel strips (GE Healthcare, Biosciences, Uppsala, Sweden) were used for samples not enriched by Zoom, whereas non-linear pH 3-10 and 3.9-5.1 IPG gel strips were used for samples enriched. The first dimension (IEF) was performed at 6,500 Vh, using a Pharmacia LKB Multiphor II system. The IPG strips were equilibrated in two steps of equilibration buffer. The first step employed 50 mM Tris-HCl buffer, pH 6.8, 6 M urea, 30% glycerol, 1% SDS and 1% DTT, while 2.5% iodoacetamide replaced DTT in the second step. The IPG strips were then applied to the second dimension 14% T SDS polyacrylamide gels (100x80x1.5mm). Electrophoresis of the mini-gel was performed in a Hoefer system at 20 mA for two hours at room temperature. After electrophoresis, proteins were visualized by CBR-250 staining.

### Gel Scanning and Image Analysis

Gels were scanned using an ImageScanner II (GE Healthcare, Uppsala, Sweden). The ImageMaster™ (GE Healthcare, Uppsala, Sweden) was used for computer analysis.

### Tryptic In-gel Digestion

Protein spots were excised and transferred to 0.5 ml microfuge tubes. Fifty µl of 0.1 M $NH_4HCO_3$ in 50% acetonitrile was added. The gel was incubated 3 times for 20 minutes at 30°C. The solvent was discarded and gel particles were dried completely by Speed Vac. Reduction and alkylation was performed by swelling the gel pieces in 50 µl buffer solution (0.1 M $NH_4HCO_3$, 10 mM DTT and 1 mM EDTA) and incubating at 60°C for 45 minutes. After cooling, the excess liquid was removed and quickly replaced by the same volume of freshly prepared 100 mM iodoacetamide in 0.1 M $NH_4HCO_3$ solution. The reaction was incubated at room temperature in the dark for 30 minutes. The iodoacetamide solution was removed and the gel pieces were washed with 50% acetonitrile in water, 3 times for 10 minutes each time, and the gel pieces were completely dried. Aliquots of trypsin (Promega Corporation, WI, USA) (1 µg trypsin /10 µl 1% acetic acid) were prepared and stored at -20°C Fifty µl of digestion buffer (0.05 M Tris HCl, 10% acetonitrile, 1 mM $CaCl_2$, pH 8.5) and 1 µl of trypsin were added to the gel pieces. After incubating the reaction mixture at 37°C overnight, the digestion buffer was removed and saved. The peptides were then extracted from the gel pieces by adding 60 µl of 2% freshly prepared trifluoroacetic acid and incubating for 30 minutes at 60°C. The extract and the saved digestion buffer were finally pooled and dried.

### Protein Identification by LC/MS/MS

LC/MS/MS analyses were carried out using a capillary LC system (Waters) coupled to a Q-TOF mass spectrometer (Micromass, Manchester, UK) equipped with a Z-spray ion-source working in the nanoelectrospray mode. Glu-fibrinopeptide was used to calibrate the instrument in MS/MS mode. The

tryptic peptides were concentrated and desalted on a 75 µm ID x 150 mm C18 PepMap column (LC Packings, Amsterdam, The Netherlands). Eluents A and B were 0.1% formic acid in 3% acetonitrile and 0.1% formic acid in 97% acetonitrile, respectively. Six µl of sample was injected into the nanoLC system, and separation was performed using the following gradient: 0 min 7% B, 35 minutes 50% B, 45 minutes 80% B, 49 minutes 80% B, 50 minutes 7% B, 60 minutes 7% B. The database search was performed with ProteinLynx screening SWISS-PROT and NCBI. For some proteins that were difficult to find, the Mascot search tool available on the Matrix Science site screening NCBInr was used.

### Glycoprotein Gel Staining

After two-dimensional electrophoresis, the gel was immersed in fixing solution (50% methanol and 5% acetic acid) with gentle agitation at room temperature for 45 minutes. This fixation step was repeated overnight and washed twice with 3% glacial acetic acid at room temperature for 20 minutes. The gel was then incubated in oxidizing solution (periodic acid in 3% acetic acid) at room temperature for 30 minutes and then washed twice with 3% glacial acetic acid at room temperature for 20 minutes. The gel was stained with freshly prepared Pro-Q Emerald 300 Staining Solution (Invitrogen, Carlsbad, CA, USA) in the dark for 2 hours at room temperature. Finally, the gel was washed again twice before visualizing using Ettan DIGE Imager (GE Healthcare).

### Phosphoprotein Gel Staining

After two-dimensional electrophoresis, the gel was fixed, washed with ultrapure water and incubated in Pro-Q Diamond phosphoprotein gel stain (Invitrogen, Carlsbad, CA, USA) in the dark for 90 minutes. The gel was destained with 20% acetonitrile, 50 mM sodium acetate, pH 4 for 30 minutes at room temperature, protected from light. After washing with ultrapure water, the stained gel was visualized on a Typhoon Imager (GE Healthcare).

## Results and Discussion

This study was aimed to investigate the changes in proteins during the dormant period of *Curcuma longa* as the role of proteins regulating its dormancy is unclear. We employed proteomic technology to study the protein expression from the day of harvest (day 0) to the commencement of sprouting (day 70) of *Curcuma longa*. The total storage period was 70 days.

### Sample Preparation for 2-D PAGE

Proteomic analysis of plant samples still has some limitations especially with regards to sample preparation. The interference of compounds such as tannins, polyphenols, lignins, alkaloids and pigments can prevent obtaining good resolution in 2-DE gels. Three solvent systems, namely acid-acetone, phenol and trichloroacetic acid, were evaluated for precipitating proteins from the rhizomes of *Curcuma* plants (data not shown). Among these solvent systems, phenol extraction is the most suitable solvent as it allows efficient protein recovery and removes non-protein components in the case of plant tissues rich in polysaccharides, lipids, and phenolic compounds (Faurobert and Pelpoir, 2007). Therefore, the phenol extraction method was selected for prior protein preparation for all experiments.

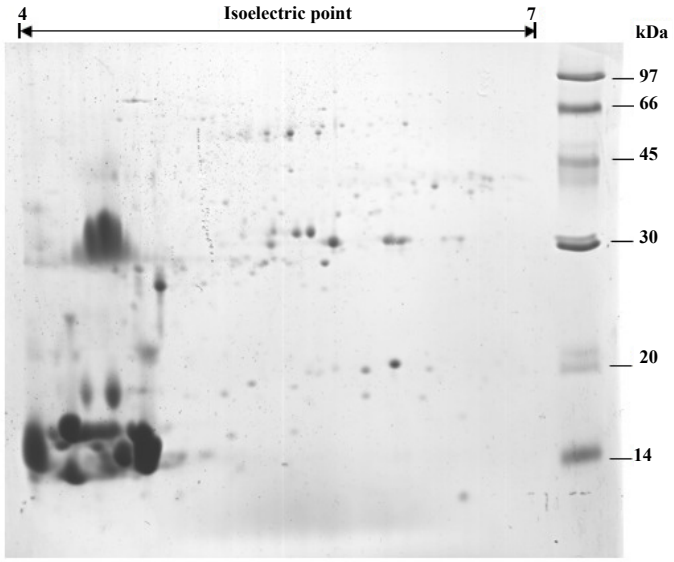


**Figure 1:** Representative two-dimensional gel electrophoresis analysis of proteins isolated from the rhizomes of *Curcuma longa* at pH range 4-7.

### Enrichment of Low Abundant Proteins Using Microscale Solution-phase Isoelectric Focusing (Zoom)

Initially, total proteins from the rhizomes of *Curcuma* plants were run on 2-DE using pH 4-7 linear strips. However, the proteomic patterns show high intensity spots in the acidic region and weak spots in the weakly acidic to basic region (Figure 1). Therefore, enrichment of the low abundant proteins in the pH range 5.4-10 was pursued using microscale solution-phase isoelectric focusing (Zoom).

### Protein Expression and Identification During the Storage Period (0-70 days) of *Curcuma Longa*

Rhizomes of *Curcuma longa* were collected from the day of harvest (day 0) until the commencement of sprouting from the buds (day 70). Protein expression during the storage period (0-70 days) of *Curcuma longa* was analyzed by two dimensional gel electrophoresis (2-DE) at day 0, day 14, day 21, day 49 and day 70. Figure 2(included as a supplementary information) shows the 2-DE patterns in the pH range 3-5.4 and Figure 3(included as a supplementary information) represents the patterns in the pH range 5.4-10 for each of the days analyzed. Most of the dark spots from the range of pH 3.0-5.4 were identified by LC/MS/MS but some spots did not match with any protein in database and could not be identified. Twenty-four spots in the range of pH 3.0-5.4 and 42 spots in the range of pH 5.4-10 patterns of *Curcuma longa* were reported in Table 1(included as a supplementary information). The spot numbers represent the spots cut from gels and identified by using LC/MS/MS.

The identified proteins were then grouped with regards to their functional classification based on the following categories: metabolism, photosynthesis, stress response, cell struc-

ture, protein synthesis, defense, transport and unannotated/function inferred. Unannotated/function inferred include a number of peptides, which have not been ascribed functions in databases. Relevant information on the functions of proteins was taken from SWISS-PROT and NCBI. Figure 4 represents the functional distribution of the identified proteins expressed in *Curcuma longa* rhizomes. Among these functional classes, 43.9% were metabolic proteins whereas proteins involved in protein synthesis, defense, stress response, photosynthesis, transport and cell structure were found to be 9.1%, 7.6%, 6.1%, 7.6%, 1.5% and 1.5%, respectively. The remaining 22.7% were annotated as unknown.

In the dormant period, less than half of all proteins identified are involved in metabolism namely, glycolysis, gluconeogen-

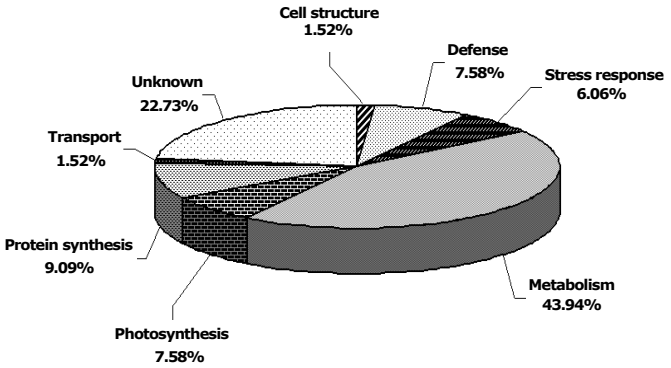


**Figure 4:** Functional distribution of identified proteins expressed in *Curcuma longa* rhizomes.

esis and starch degradation. Multiple spots were detected for proteins involved in glycolysis, namely, glyceraldehyde-3-phosphate dehydrogenase (3 forms, spot no. 25, 45, 46), fructose biphosphate aldolase (spot no. 26), enolase (2-phosphoglycerate hydratase) (3 forms, spot no. 29, 54, 55), phosphoglycerate kinase (3 forms, spot no. 32, 47, 56) and triose phosphate isomerase (spot no. 36). Two proteins involved in gluconeogenesis were detected, i.e., alcohol dehydrogenase II (spot no. 37) and alcohol dehydrogenase (spot no. 66). Two proteins involved in starch degradation were isoamylase (spot no. 38) and alpha-glucan phosphorylase, H isozyme (spot no. 52). Three proteins involved in amino acid biosynthesis were s-adenosylhomocysteine hydrolase (spot no. 48), threonine synthase chloroplast precursor TS (spot no. 50) and s-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase (spot no. 51).

Six proteins responsible for protein synthesis with major roles in controlling cell growth division and development were identified, which include 60S ribosomal protein L10 (spot no. 33), ribosomal protein subunit 2 (spot no. 34), ribosomal protein S7 (spot no. 44), ribosomal protein S18 (spot no. 65), maturase K (spot no. 10) and beta subunit of RNA polymerase (spot no. 23). There were four proteins involved in photosynthesis, namely, photosystem I assembly protein (spot no. 5), putative PSII-P protein (spot no. 20), oxygen-evolving enhancer protein 2 (spot no. 4) and putative oxygen-evolving enhancer protein 1 (spot no. 18). Ribulose 1, 5-bisphosphate carboxylase/oxygenase small subunit (spot no. 2), a protein involved in the Calvin cycle or the 3-carbon pathway, was found. There were two proteins involved in the 4-carbon pathway, i.e., NADP-dependent malic enzyme (spot no. 28) and phosphoenolpyruvate carboxylase (spot no. 53).

Sporamin A precursor (spot no. 13), the major storage protein of the tuberous roots of sweet potato, was present. Proteins involved in isoprenoid biosynthesis pathway, such as putative sesquiterpene cyclase (spot no. 9) and farnesyl pyrophosphate synthase 2 (spot no. 31), were found in the rhizomes. The expression of the enzyme for catalyzing the synthesis of catechin, leucanthocyanidin reductase (spot no. 58), was found for the first time in the rhizomes. Proteins involved in stress response were 18.3, 18.1 and 17.6 kDa class I heat shock proteins (spot no. 39, 40, 41).

### Differential Protein Expression During Dormancy and Sprouting of *Curcuma Longa*

Using ImageMaster™ computer analysis, the percent volumes of each spot at day 0, 14, 21, 49 and 70 days were determined and shown in Figure 5. The enzymes involved in carbohydrate metabolism were found in the rhizomes of *Curcuma longa*. Enzymes involved in glycolysis were present from day 0, e.g., glyceraldehyde-3-phosphate dehydrogenase (GADPH), phosphoglycerate kinase, triose phosphate isomerase and enolase, and all of these enzymes were present at day 70 when sprouting was observed. The highest level of all proteins detected was GAPDH (spot no. 25, 45), a classical glycolytic enzyme, involved in cellular energy production and has important housekeeping function. The expression levels of GAPDH were maximal at day 14 and decreased upon sprouting. Another spot (spot no. 46) was detected at lower levels. The level of another glycolytic enzyme, fructose biphosphate aldolase (spot no. 26), was also highest at day 14 and gradually decreased.

Enzymes involved in gluconeogenesis and starch degradation pathway were also detected in our analysis at day 70. This result correlates well with a study performed in which the starch content in the tubers of *D. esculenta* decreased as sprouting initiated. It showed that when a single sprout grows, it draws upon the carbohydrates in every part of the tuber simultaneously (Davis and Ross, 1984). A comparative analysis of starch and sugars during the dormant period until sprouting indicates that the total sugars present increased steadily as sprouting progressed while the starch content decreased. A similar trend was also reported in potato tubers (Copp et al., 2000). Accumulation of sugars was observed in *Lilium rubellum* bulbs during dormancy and it increased to higher levels during sprouting (Xu et al., 2006). The carbohydrates and sugar contents of *Curcuma alismatifolia* Gagnep were studied during their dormant period (Ruamrungsri et al., 2001). The hydrolysis of starch in the rhizome and storage roots in the middle of the dormant period was observed, however total soluble sugars did not increase. It was therefore suggested that active utilization of carbohydrates was essential for shoot growth in both rhizome and storage roots during dormancy.

Proteins involved in amino acid biosynthesis, namely, S-adenosyl-Lhomocysteine (SAH) hydrolase (spot no. 48), threo-

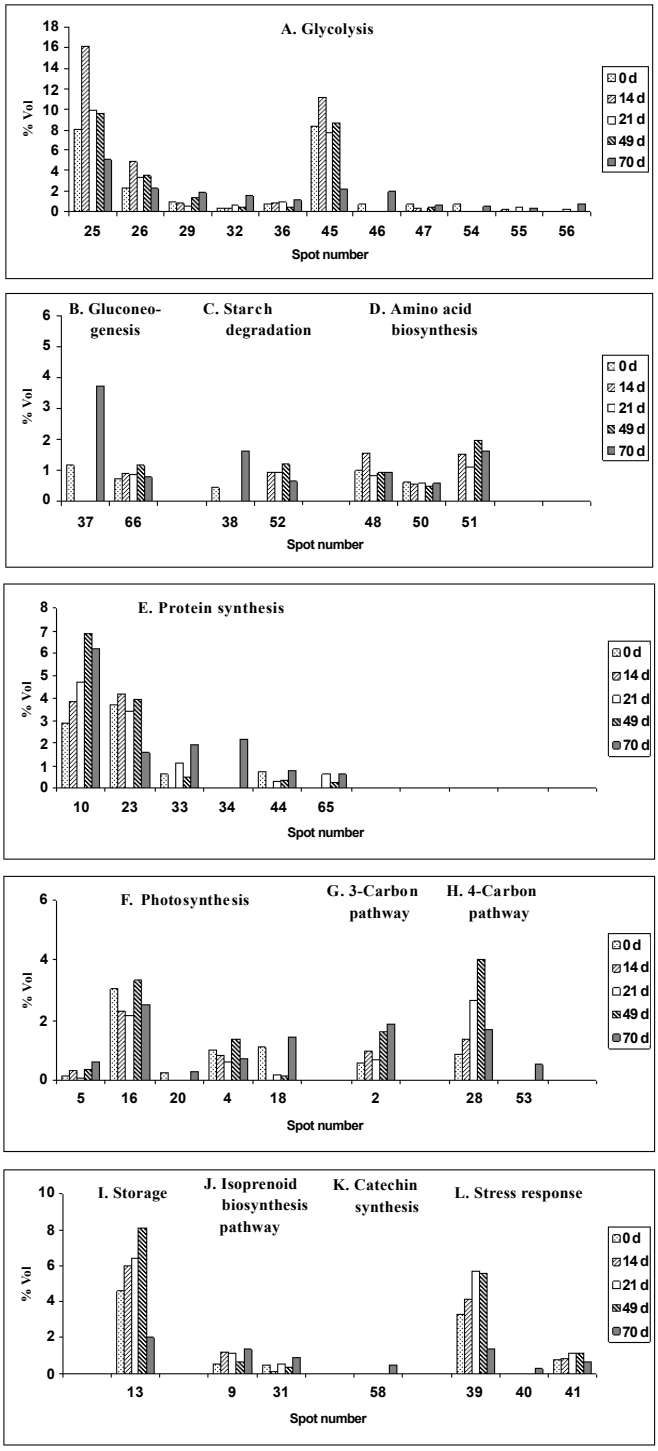


**Figure 5:** The quantifications of the proteins involved in glycolysis (A), gluconeogenesis (B), starch degradation (C), amino acid biosynthesis (D), protein synthesis (E), photosynthesis (F), 3-carbon pathway or calvin cycle (G), 4-carbon pathway (H), storage protein (I), isoprenoid biosynthesis (J), catechin synthesis (K) and stress response (L).

nine synthase chloroplast precursor TS (spot no. 50) and S-methyltetrahydropterpyl-triglutamate-homocysteine methyltransferase (spot no. 51), were prominent during dormancy and early sprouting. The new caffeine biosynthetic pathway in tea leaves was reported on the utilization of adenosine released from the S-adenosyl-L-methionine (SAM) cycle (Koshiishi et al., 2001). SAM is converted to SAH, which in turn is hydrolyzed to L-homocysteine and adenosine. High SAH hydrolase activities in cell-free extracts from young tea leaves were detected.

Maturase K (spot no. 10) and the beta subunit of RNA polymerase (spot no. 23) were detected as well in relatively high amounts during storage, but the levels started to decrease upon sprouting (day 70). These proteins are significant for controlling protein synthesis along with the other ribosomal proteins detected. The photosynthetic proteins were found from day 0, i.e., photosystem I assembly protein ycf4 (spot no. 5), plastocyanin-like (spot no. 16), oxygen-evolving enhancer protein 1 and 2 (spot no. 18 and 4). Only one protein involved in the Calvin cycle or the C-3 pathway, i.e., ribulose 1, 5-bisphosphate carboxylase/oxygenase small subunit (spot no. 2) was detected, and the levels increased from day 0 to day 70. Differential expression was observed for proteins involved in C-4 pathway. NADP-dependent malic acid (spot no. 28) was found from day 0 to day 70 but phosphoenolpyruvate carboxylase (spot no. 53) was present at day 70. Usually plants in the tropical areas such as sugar cane, rice and maize are called C4 grasses. The molecular basis for C4 differentiation is still poorly understood. Differential protein accumulation and activities between mesophyll and the bundle sheath chloroplast of maize and sorghum (both NADP-Malic enzyme (ME) type C4) have been studied using various low throughput techniques (Hatch and Slack, 1966).

The proteins involved in isoprenoid biosynthesis such as putative sesquiterpene cyclase (spot no. 9), farnesyl pyrophosphate synthase 2 (spot no. 31), were present in the rhizome during the whole storage period. The content of bisabolane-type sesquiterpenes in volatile oil was found in high amount for *Curcuma longa* (Zeng et al., 2007). The key step in terpenoid biosynthesis is the conversion of acyclic prenyldiphosphates to terpenoid compounds by specific terpenoid synthase (cyclases) (Shen et al., 2000). Sesquiterpene cyclase, a branch point enzyme in the general isoprenoid pathway for the synthesis of phytoalexin capsidiol, was induced in detached leaves of *Capsicum annuum* (pepper) by UV treatment. The inducibility of cyclase enzyme activities paralleled the absolute amount of cyclase protein(s) of pepper immunodetected by monoclonal antibodies raised against tobacco sesquiterpene cyclase (Back et al., 1998).

Leucoanthocyanidin reductase (spot no. 58), the NmrA-type oxidoreductase family or isoflavone reductase subfamily was first found by our group in the rhizome of sprouting period of *Curcuma longa*. This protein catalyzes the synthesis of catechin from 3,4-cis-leucocyanidin. Cathechins are polyphenolic antioxidant plant metabolites. These compounds are abundant in teas derived from the tea-plant *Camellia sinensis* as well as in some cocoas and chocolates made from the seeds of *Theobroma cacao* (Punyasiri et al., 2004).

Heat shock proteins were found as sHsp family with molecular weight 18.3, 18.1 and 17.6 kDa (spot no. 39, 40, 41). In plants, sHsps form a more diverse family than other Hsps/chaperones with respect to sequence similarity, cellular location and functions (Wang et al., 2004; Waters et al., 1996; Vierling, 1991). sHsp functions by preventing aggregation and stabilizing non-native proteins. Under normal growth conditions, most sHsps

cannot be detected in the vegetative tissues, but are rapidly produced in response to heat. Increasing the temperature to approximately 10-15ºC above the optimal growth temperature, which is usually in the sub-lethal range, induces the heat shock response.

Vegetative storage organs such as bulbs, tubers, corms, rhizomes and bark act as sinks for soluble nitrogen compounds (mainly amino acids) generated from the leaf proteins when the plant enters a senescing phase (Van Damme et al., 2000). These storage organs become a source of nitrogen when the plant resumes growth after a resting or dormancy period. There are reports of increased 1-2% dry weight in cassava and up to 10% dry weight in yam bean (Shewry, 2003). Classical examples of storage proteins are the tuber storage proteins from potato (*Solanum tuberosum*) and sweet potato (*Ipomoea batatas*), which are commonly named as patatin and sporamin, respectively (Mignery et al., 1984; Maeshima et al., 1985). From our result, sporamin, the major storage protein of the tuberous roots of sweet potato, was highly expressed during the storage period in *Curcuma longa*, with the highest level at day 49. Sporamin was reported to have antioxidant activity, acting as a dehydroascorbate reductase and monodehydroascorbate reductase, and that this was associated with intermolecular thiol/disulfide exchange (Hou and Lin, 1997).

**Post-translational Modifications of Proteins During Storage Period (0-70 days) of *Curcuma Longa***

The presence of post-translational modifications in proteins in the 70-day *Curcuma longa* rhizomes can be detected by glycoprotein (Figure 6A, C) and phosphoprotein (Figure 6B, D) staining of the 2-DE gels of proteins enriched at the acidic and basic ranges, respectively. Some proteins enriched at pH 3.0-5.4 that were positive for glycosylation included ribulose carboxylase/oxygenase small subunit (spot no. 2), photosystem I assembly protein ycf4 (spot no. 5), peptidyl-prolyl cis trans isomerase-like (spot no. 6), actin (spot no. 8), putative sesquiterpene cyclase (spot no. 9), F-box protein interaction domain (spot no. 12), sporamin A precursor (spot no. 13), peptidase M (spot no. 15) and beta subunit of RNA polymerase (spot no. 23) as shown in Figure 6A. Most of those spots were also positive for phosphorylation except for actin (spot no. 8), putative sesquiterpene cyclase (spot no. 9) and beta subunit of RNA polymerase (spot no. 23), with the presence of phosphorylation of plastocyanin-like protein (spot no. 16) as shown in Figure 6B.

For enriched pH 5.4-10 proteins shown in Figure 6C, six proteins were found to be positive with glycoprotein staining, which are ribosomal protein subunit E (spot no. 34), vacuolar ATP synthase (spot no. 35), alcohol dehydrogenase II (spot no. 37), isoamylase (spot no. 38), glyceraldehyde-3-phosphate-dehydrogenase (spot no. 45, 46), and 5-Methyltetrahydropteroytriglutamatehomocysteine methyltransferase (spot no. 51). Seven proteins in Figure 6D, glyceraldehyde-3-phosphate-dehydrogenase (spot no. 25, 45), fructose biphosphate aldolase

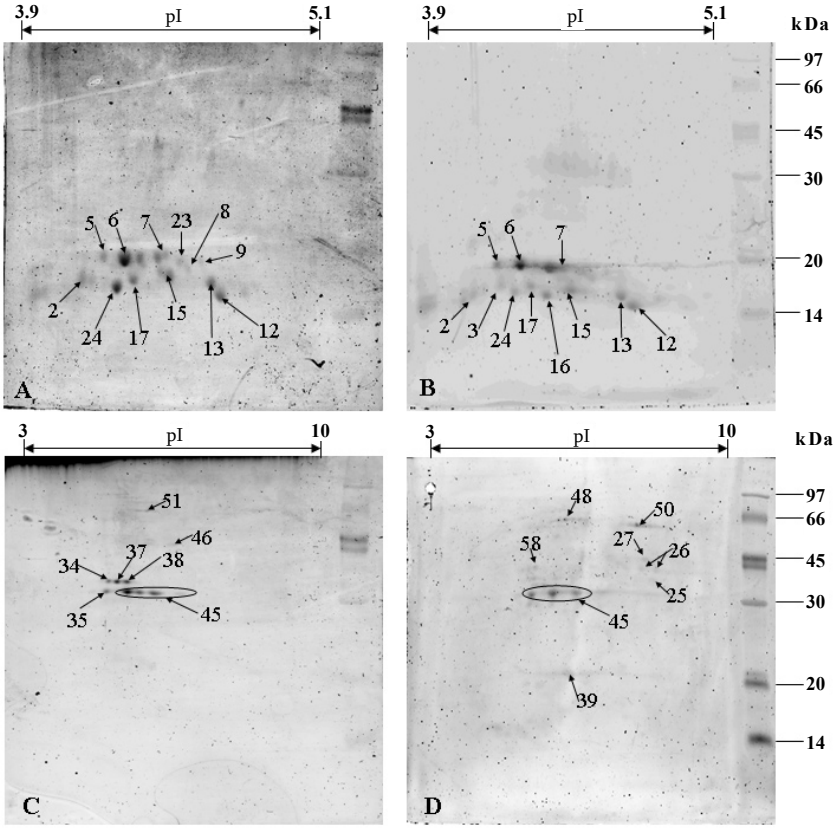


**Figure 6:** Identification of post-translational modifications present in *Curcuma longa* rhizomes. The 2-DE gels of the enriched pH 3.0-5.4 proteins and the enriched 5.4-10 proteins from 70 days of the *Curcuma longa* rhizomes were stained with Pro-Q Emerald 300 Staining Solution for glycoprotein detection (A, C) and Pro-Q Diamond phosphoprotein gel stain (B, D), respectively. The identity of each labeled spot is listed in Table 1.

(spot no. 26), formate dehydrogenase 1 (spot no. 27), 18.3 kDa class I heat shock protein (spot no. 39), Sadenosylhomocysteine hydrolase (spot no. 48), threonine synthase chloroplast precursor TS (spot no. 50) and leucoanthocyanidin reductase (spot no. 58) were found to be positive with phosphoprotein staining.

## Conclusions

For the rhizomes of *Curcuma longa*, visible sprouting was observed within 70 days after harvest. The first proteomic patterns during the dormancy period until sprouting (day 70) of this plant were studied. Two forms of glyceraldehyde-3-phosphate dehydrogenase were present at high levels as isotype and only one form showed both glycosylated and phosphorylated pattern. Sporamin, the major storage protein of the tuberous roots of sweet potato was highly expressed in the dormant period and lower expression seen in the sprouting period. The expression of putative sesquiterpene cyclase and farnesyl pyrophosphate synthase 2, involved in the isoprenoid biosynthesis, were found in the dormant period. The enzyme for catalyzing the synthesis of catechin, leucoanthocyanidin reductase, was found for the first time in the *Curcuma longa* rhizome. Further investigation of other species will be useful to determine species-specific questions.

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