



Detection of superoxide dismutase (Cu–Zn) isoenzymes in leaves and pseudobulbs of *Bulbophyllum morphologlorum* Kraenzl orchid by comparative proteomic analysis

Pattana S. Huehne^a, Kisana Bhinija^a, Chantragan Srisomsap^{b,*}, Daranee Chokchaichamnankit^b, Churat Weeraphan^b, Jisnuson Svasti^{b,c}, Skorn Mongkolsuk^{a,c}

^a Laboratory of Biotechnology, Chulabhorn Research Institute, Bangkok, 10210, Thailand

^b Laboratory of Biochemistry, Chulabhorn Research Institute, Bangkok, 10210, Thailand

^c Applied Biological Sciences Program, Chulabhorn Graduate Institute, Chulabhorn Royal Academy, Bangkok, 10210, Thailand



ARTICLE INFO

Keywords:

Bulbophyllum
Orchid
Proteomics
Stress response
Enzymatic antioxidant
Superoxide dismutase (Cu–Zn)

ABSTRACT

Typically, biological systems are protected from the toxic effect of free radicals by antioxidant defense. Extracts from orchids have been reported to show high levels of exogenous antioxidant activity including *Bulbophyllum* orchids but so far, there have been no reports on antioxidant enzymes. Therefore, differences in protein expression from leaves and pseudobulbs of *Bulbophyllum morphologlorum* Kraenzl and *Dendrobium Sonia* Earsakul were studied using two-dimensional gel electrophoresis and mass spectrometry (LC/MS/MS). Interestingly, the largest group of these stress response proteins were associated with antioxidant defense and temperature stress, including superoxide dismutase (Cu–Zn) and heat shock protein 70. The high expression of this antioxidant enzyme from *Bulbophyllum morphologlorum* Kraenzl was confirmed by activity staining on native-PAGE, and the two Cu/Zn-SODs isoenzymes were identified as Cu/Zn-SOD 1 and Cu/Zn-SOD 2 by LC/MS/MS. The results suggested that *Bulbophyllum* orchid can be a potential plant source for medicines and natural antioxidant supplements.

1. Introduction

The effect of oxidative stress and the process of autoxidation cause human diseases such as cardiovascular diseases, aging, cancers and diabetes [1]. Many antioxidants have been synthesized and used to prevent the process, but sometimes produced side effects [2]. As a result, natural antioxidants have been obtained from plants as potential medicines to prevent and/or treat such diseases [3]. The search for safe antioxidants from plants still continues. One of the most important enzymatic antioxidants that constitute the first line of antioxidant barrier against reactive oxygen species-induced damages is superoxide dismutase (SOD) [4,5]. Based on the catalytic metal ions at the active sites, SODs are classified into three distinct groups: Fe, Mn and Cu/Zn-SOD [6]. Diminished activities of SODs have been reported in various physiological and pathological conditions e.g. cancer, inflammatory diseases, aging and skin disorders. To date, several studies suggest that SODs are useful agents for prevention or treatment of various skin disorders, especially in melanoma cancer and skin inflammation. In

plants, superoxide dismutases may contain different catalytic metal ions at the active site: Cu/Zn, Mn and Fe. The differences in type, number and distribution of metalloenzymes depend on the species, stage of development and environment [7–11]. In addition, SODs with the same metal cofactor can change roles in different species [12]. Iron-SODs are the oldest group of ubiquitous enzymes, found in chloroplasts and cytoplasm [13,14]. Manganese-SODs are present in mitochondria and peroxisomes [15]. The Cu/Zn-SODs were reported to compose of two subunits with a combination of Cu and Zn atoms, respectively [16]. They are found in the chloroplasts, cytosol, peroxisomes and the apoplast [17–19]. In recent years, the SODs have been reported to play a role in plant protection against abiotic and biotic stress [20].

The *Orchidaceae* is a widely distributed flowering plant family, found in all types of habitats, and includes terrestrial, saprophytic, and epiphytic orchids. The *Bulbophyllum* orchid, an epiphyte, has some 1000 species in Africa and Asia, with the latter being mainly in China, Nepal, Sikkim, Bhutan, India, Burma, Thailand, Laos, and Vietnam [21]. Thailand has 154 known species of *Bulbophyllum*, making it the second

* Corresponding author.

E-mail addresses: pattana@cri.or.th (P.S. Huehne), kisana@cri.or.th (K. Bhinija), chantragan@cri.or.th (C. Srisomsap), daranee@cri.or.th (D. Chokchaichamnankit), cweeraphan@gmail.com (C. Weeraphan), jisnuson@cri.or.th (J. Svasti), skorn@cri.or.th (S. Mongkolsuk).

<https://doi.org/10.1016/j.bbrep.2020.100762>

Received 12 December 2019; Received in revised form 26 February 2020; Accepted 27 February 2020

2405-5808/© 2020 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

most prevalent orchid genus after *Dendrobium* orchids [22]. *Dendrobium* and *Bulbophyllum* species have a long history and are commonly used as traditional Chinese medicines (TCM) in Asian countries [21,23–25]. Two known *Bulbophyllum* species, *B. kwangtungense* Schlecht (Shi dou-lan) [21,26] and *B. odoratissimum* Lindl [27]. are used as medicinal orchids in the treatment of tuberculosis, chronic inflammation, and fever reduction [23,24]. Several reports have described the phytochemical constituents and biological effects of the chemical compounds extracted from the entire plant or plant parts (leaf, pseudobulb, or root) of *Bulbophyllum* used for various disease treatments [24]. The extracts from some orchids show high levels of exogenous antioxidant activity such as flavonoids in the leaves of *Rhynchosyris retusa* [28], and in the stems of *Bulbophyllum kaitense* [29], as well as the polyphenolics in the stems of *Vanda cristata* [28]. *Dendrobium nobile* was reported to be a potential source of antioxidants [30]. Orchids are therefore considered as good sources for antioxidants, but there is still no report on enzymatic antioxidants from *Bulbophyllum* orchids.

Proteomic techniques, using two-dimensional gel electrophoresis and nanoLC-mass spectrometry, is used worldwide to identify proteins from biological samples including plants and animals. Recently, proteomic studies of orchids have been reported to study various aspects, for example: the generation of the protocorm-like body of *Vanilla planifolia* Jacks. ex Andrews [31,32]; the browning in leaf culture of *Phalaenopsis* [33]; the pollination of the flower of *Ophrys* spp. [34], *Cymbidium ensifolium* (L.) Sw [35]. and *Dendrobium chrysanthum* [36]; the symbiotic reaction between fungi and the seeding of *Oncidium sphacelatum* Lindl [37,38]. and *Dendrobium officinale* Kimura and Migo herb [39,40]; the succinyl-proteome profile of the entire plant of *Dendrobium officinale* Kimura et Migo herb [41]; the adaptive drought strategies of *Cymbidium sinense* and *C. tracyanum* [42]; and the adaptive development of a tolerant mechanism to heavy metals by mycorrhizal *Bipinnula fimbriata* [43]. But there are still no data available in terms of the major proteins produced in the leaves and pseudobulbs of *Bulbophyllum* orchid.

Since our previous work (unpublished data) suggested that ethanol extracts of *Bulbophyllum morphologlorum* Kraenzl. (semi-epiphytic orchids) and *Dendrobium Sonia* Earsakul (epiphytic orchid) showed significant DPPH radical scavenging assay, as determined by the method of van Amsterdam et al. [44], we decide to investigate the endogenous enzymatic antioxidant activity of leaves and pseudobulbs of these orchids. Thus, comparative protein expression of *Bulbophyllum morphologlorum* Kraenzl and of *Dendrobium Sonia* Earsakul was studied by two-dimensional electrophoresis (2-DE) and nanoLC/MS/MS technology. In the present work, information was obtained on the differential expression of proteins and protein functions. The proteins involved in stress response were found in the highest amounts in *Bulbophyllum* orchid. SOD activity was detected by staining on native-PAGE and finally identified as Cu/Zn-SOD by nanoLC/MS/MS.

2. Materials and Methods

2.1. Plant materials and phenol protein extraction

Three-year-old *Bulbophyllum morphologlorum* Kraenzl. derived from seedlings were grown in a greenhouse at the Chulabhorn Research Institute, and *Dendrobium Sonia* Earsakul was purchased from the Chatuchak Sunday Market, Bangkok, Thailand. Ten grams of fresh leaf and pseudobulb samples were collected separately from mature orchids, and then immediately ground to a fine powder in liquid nitrogen prior to protein extraction with 50 mL of extraction buffer A (0.1 M Tris-HCl pH 8.8, 100 mM KCl, 0.4% 2-mercaptoethanol, 0.7 M sucrose), and the supernatant transferred to a new tube. After addition of 1 volume of extraction buffer B, consisting of the same buffer A with the addition of 2 mM phenylmethanesulfonyl fluoride (PMSF) and 50 mM ethylenediaminetetraacetic acid (EDTA) as protease inhibitors [45], the solution was mixed using a vortex, left at 4 °C for at least

30 min and centrifuged for 20 min, 4000 g at 4 °C. The supernatant was removed into a new tube and kept at 4 °C, and the pellet was extracted one more time using the same extraction buffer. The supernatant was combined with the first extraction and added with an equal volume of water-saturated phenol. The solution was mixed vigorously and kept on ice for 1 h, the solution was centrifuged for 20 min, 8000 g at 4 °C and the phenol phase was transferred to a new tube. The same phenol extraction was repeated one more time. Pooled phenol phase was added with 5 vol of 0.1 M of ammonium acetate in methanol and left overnight at –20 °C for protein precipitation. The sample was centrifuged as above and the protein pellet was dissolved immediately in cold water, sonicated for 3 min and then added with 9 vol of cold acetone. The solution was left at –20 °C for about 4 h to precipitate protein and centrifuged as above. The protein pellet was removed, dried and stored at –80 °C.

2.2. Two-dimensional gel electrophoresis (2-DE)

The protein pellet was resuspended in IEF buffer (7 M urea, 2 M thiourea, 4% CHAPS, 2% triton X-100, 100 mM DTT, 1% ampholytes pH 3–10, and 0.005% bromophenol blue). Then, pre-cast, 7 cm immobilized pH gradient strips (IPG strip), with a pH 4–7 linear gradient (GE Healthcare, UK), were loaded with 300 µg of protein in IEF buffer for each IPG strip, and rehydrated overnight. The 1st dimension was run in an EttanIPGphor II IEF Unit (GE Healthcare, UK) with these conditions: step 1, hold at 300 V for 30 min; step 2, gradient at 1000 V for 30 min; step 3, gradient at 5000 V for 90 min; and step 4, hold at 5000 V for 12–36 min. After the 1st dimension, proteins were reduced by incubating the IPG strips with 1% w/v DTT in equilibration buffer (6 M urea, 30% w/v glycerol, 2% SDS, and 50 mM Tris-HCl, pH 8.8), and alkylated with 2.5% w/v iodoacetamide in equilibration buffer (6 M urea, 30% w/v glycerol, 2% SDS, and 50 mM Tris-HCl, pH 8.8) [46]. The IPG strips were embedded within molten agarose directly on top of a 1.5 mm × 10 cm × 10.5 cm SDS-PAGE gel (4% stacking gel, 12.5% separating gel). Separation in the 2nd dimension involved SDS-PAGE with a constant current of a 12 µA/IPG strip for 3 h per gel. The protein spots were visualized by staining with 0.1% Coomassie brilliant blue R-250. The gel images were captured using the LabScan Image Scanner II software (GE Healthcare, UK), and the total protein spots were analyzed using the ImageMaster 2D Platinum 6.0 software (GE Healthcare, UK) by matching and comparing the differences in the % volume of the protein spots. The experiments were studied independently in triplicate. The protein spots that showed significant difference in volume ratio ($P \leq 0.05$) were selected for further analysis using mass spectrometry.

2.3. Protein identification using mass spectrometry analysis

The selected protein spots from the 2-DE gels were excised and destained with 0.1 M NH_4HCO_3 and 50% acetonitrile. The disulphide bonds were reduced with 0.1 M NH_4HCO_3 , 10 mM DTT, and 1 mM EDTA, alkylated with 100 mM iodoacetamide in 0.1 M NH_4HCO_3 and digested with trypsin. Liquid chromatography tandem-mass spectrometry (LC-MS/MS) analyses were carried out on a capillary LC system coupled to a Quadrupole-Time of flight tandem mass spectrometer (Waters Micromass, UK) equipped with a Z-spray ion-source working in the nanoelectrospray mode. Glu-fibrinopeptide was used to calibrate the instrument in the MS/MS mode, and tryptic peptides were concentrated and desalted on a 75 µm ID × 150 mm C18 PepMap column (LC Packings, the Netherlands). Eluents A and B consisted of 0.1% formic acid in 97% water and 3% acetonitrile, and 0.1% formic acid in 97% acetonitrile, respectively. A 6 µL sample was injected into the nanoLC system, and the separation was performed with the following gradient: 0 min 7% B, 35 min 50% B, 45 min 80% B, 49 min 80% B, 50 min 7% B, and 60 min 7% B.

A database search using SWISS-PROT (<http://www.ebi.ac.uk/>

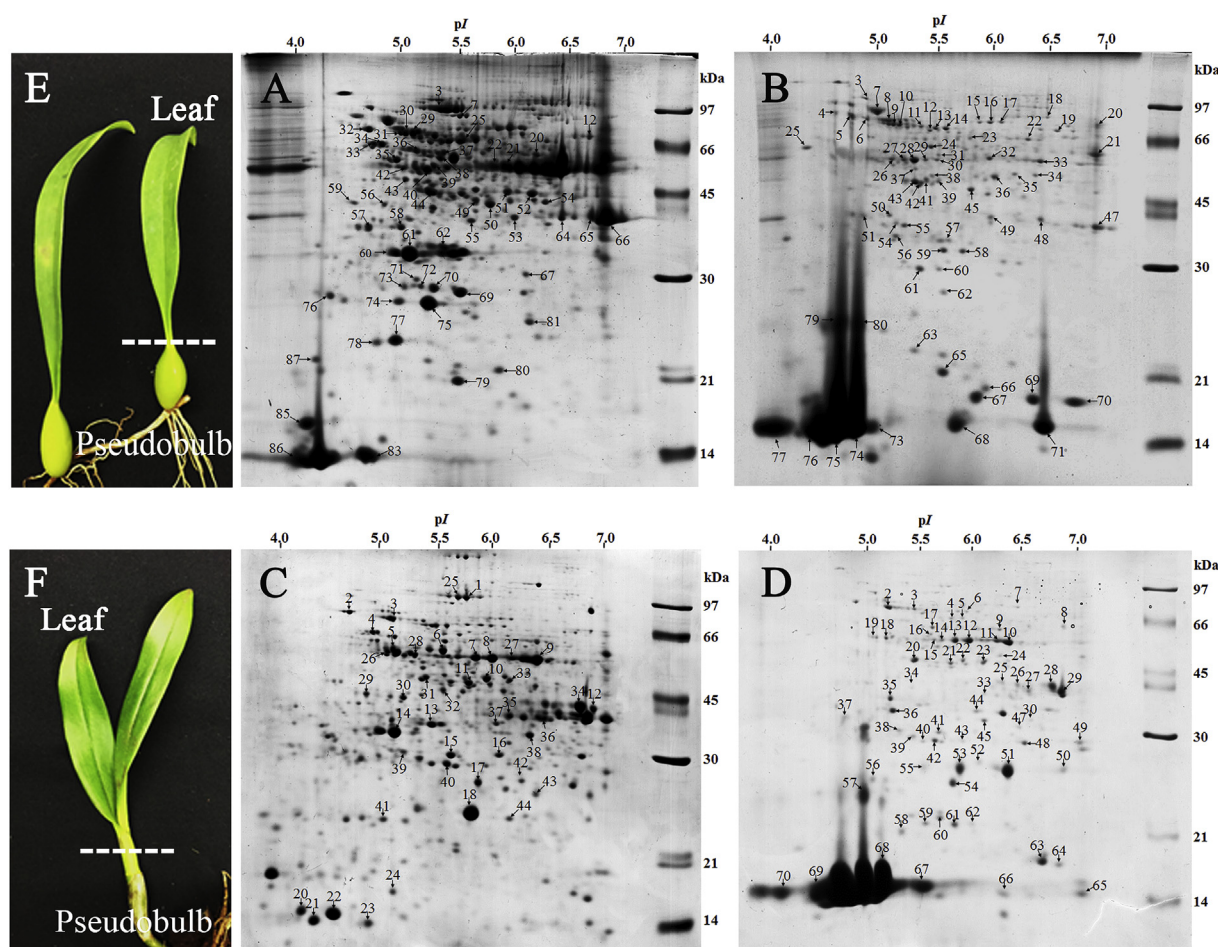


Fig. 1. Proteomic profiles of leaves (A) and pseudobulbs (B) of *Bulbophyllum morphologlorum* Kraenzl and of leaves (C) and pseudobulbs (D) of *Dendrobium Sonia Earsakul*. The 2-D electrophoresis was obtained using 300 μ g phenol extracted proteins from both tissues of the orchids and 7 cm IPG with pH from 4 to 7 was used for the 1st dimension. E is Leaf and pseudobulb tissue of *Bulbophyllum morphologlorum* Kraenzl while F is Leaf and pseudobulb tissue of *Dendrobium Sonia Earsakul*.

uniprot/) and NCBI (<http://www.ncbi.nlm.nih.gov/protein/>) was performed with ProteinLynx (Waters Micromass, Manchester, UK). The Mascot search tool, available on the Matrix Science site (<http://www.matrixscience.com>), was used for some proteins which were not found in the previous databases [47]. The search parameters were used as follow: Database, Swiss-Prot; taxonomy, Viridiplantae (Green Plants), peptide mass tolerance was 1.2 Da, MS/MS ion mass tolerance was 0.6 Da, allowance was set to 1 missed cleavage, trypsin was set as the used enzyme and the peptide charge limit was set at 2+ and 3+. The identification of protein was analyzed by using p -value ≤ 0.05 and Mascot score > 30 being considered as promising hits. Our criteria followed those of Kristiansen et al. [48], for example one matched peptide composed of at least 8 amino acids and a sequence tag of at least 3 amino acids would be considered as a good y-ion series. The peptide and Mascot score for proteins containing one matched peptide should be greater than 30. Protein function was obtained from the UniProt website (<http://www.uniprot.org>) [49]. Two-way statistical analysis of variance with Tukey's Honest Significant Difference post-hoc analysis was performed. Values were considered to indicate a statistically significant at $p < 0.05$ [50].

2.4. Protein-protein interaction analysis

STRING (the Search Tool for Retrieval of Interacting Genes/Proteins) database v 9.0 (string-db.org) was employed to obtain the interaction network. The confidence score was defined by STRING and the interaction confidence was calculated. The interaction network was

constructed with a high confidence score > 0.4 . Cytoscape software (<http://www.cytoscape.org>) was used as a tool to visualize the protein-protein interaction network.

2.5. Protein precipitation by ammonium sulfate

Three grams of fresh leaf and pseudobulb samples from *Bulbophyllum morphologlorum* Kraenzl and *Dendrobium Sonia Earsakul* were collected from mature orchids, and then immediately ground separately to a fine powder in liquid nitrogen and left in 5 mL of extraction buffer (0.1 M NaCl, 20 mM phosphate buffer pH 7.2) at 4 °C. The mixture was stirred at 4 °C overnight and later centrifuged at 10,178 $\times g$ for 30 min at 4 °C and the supernatant was collected. Then ammonium sulfate was added to the supernatant to 90% saturation, and the mixture was left overnight at 4 °C. Precipitated material was obtained by centrifugation (15,904 $\times g$, 30 min, 4 °C). The precipitate was dissolved in 400 μ L deionized water, and dialyzed against 1000 mL of 20 mM phosphate buffer pH 7.2 (with 4 changes of the fresh buffer) over 18 h at 4 °C. The dialyzed material was then dried using speed-vac. The amount of protein was calculated by the Bradford assay [51].

2.6. Native polyacrylamide gel electrophoresis of SOD activity

The native-PAGE using 12.5% (w/v) polyacrylamide was prepared. The protein sample was dissolved in sample buffer without boiling. The gel was stained for SOD activity using the Chopra method [52]. Thirty micrograms of extracted proteins from leaves and pseudobulb of both

Table 1
Identified proteins of *Bulbophyllum morphologorum* Kranzl. (BM) and *Dendrobium Sonia Earsakul* (DE) by LC-MS/MS.

Spot no.	Protein Identification	Accession no.	MASCOT score	% Coverage	(MW/pI) ^a Theoretical	(MW/pI) ^b Experimental	Functions
<i>Bulbophyllum morphologorum's</i> leaves							
A3	TMV resistance protein N-like (Eucalyptus grandis)	gi 702444611	38	2%	39.64/7.53	97.00/5.4	Stress response
A7	Hydroquinone glucosyltransferase (Eucalyptus grandis)	gi 702327425	45	3%	53.82/6.10	94.93/5.5	Stress response
A12	Auxin-binding protein ABP19a (Fragaria vesca subsp. vesca)	gi 470105207	31	3%	22.98/5.90	74.26/6.6	Stress response
A20	ZG10 (Protein amino acid glycosylation) (Pisum sativum)	gi 37813069	45	3%	28.26/7.25	61.16/6.3	Glycolysis and gluconeogenesis
A21	Centromeric histone H3 (Brassica juncea)	gi 134152527	42	4%	19.48/11.60	58.73/5.8	Cellular communication and signal transduction
A22	Gastrodin-4B (Gastrodia elata)	gi 62479957	86	11%	18.21/8.58	58.73/5.7	Stress response
A25	Pyruvate, phosphate dikinase (Arabidopsis thaliana)	gi 79475768	45	7%	95.332/5.36	74.27/5.5	Glycolysis and gluconeogenesis
A29	Heat shock cognate 70 kDa protein 2 (Zea mays)	gi 195616644	165	9%	71.09/5.06	78.40/5.2	Stress response
A30	Heat shock protein 70 (Camellia sinensis)	gi 189380223	63	7%	75.07/5.54	78.40/5.1	Stress response
A31	Heat shock cognate 70 kDa protein 2 (Zea mays)	gi 226500092	52	3%	71.09/5.15	78.40/5.0	Stress response
A32	Heat shock protein 70 (Cucumis sativus)	gi 1143427	192	10%	75.37/4.99	78.76/4.8	Stress response
A33	RuBisCO large subunit-binding protein subunit alpha, chloroplastic (Fragment) (Brassica napus)	gi 289365	86	8%	57.66/4.84	70.13/4.8	Photosynthesis and photorespiration
A34	RuBisCO large subunit-binding protein subunit alpha, chloroplastic (Pisum sativum)	gi 219902505	65	3%	61.94/5.15	70.13/4.8	Photosynthesis and photorespiration
A35	V-type proton ATPase subunit B1 (Vitis vinifera)	gi 225428086	197	17%	54.25/5.04	61.16/5.0	Cellular communication and signal transduction
A36	RuBisCO large subunit-binding protein subunit beta (Pisum sativum)	gi 2506277	56	6%	62.94/5.85	64.79/5.2	Photosynthesis and photorespiration
A37	4-hydroxy-tetrahydrodipicolinate synthase, chloroplastic (Coix lacryma-jobi)	gi 300572573	37	1%	41.05/6.84	62.37/5.5	Amino acid metabolism
A38	ATP synthase subunit alpha, chloroplastic (Lotus japonicus)	gi 13518443	32	9%	55.75/5.22	59.95/5.3	Photosynthesis and photorespiration
A39	ATP synthase subunit beta, chloroplastic (Eucalyptus globulus subsp. Globulus)	gi 60460816	31	11%	53.69/5.29	56.31/5.3	Photosynthesis and photorespiration
A40	Enolase 1 (Zea mays)	gi 162458207	202	6%	48.03/5.20	53.89/5.3	Glycolysis and gluconeogenesis
A42	Nicotianamine synthase (Ricinus communis)	gi 255585344	42	10%	75.81/7.29	55.10/5.0	Stress response
A43	DEAD-box ATP-dependent RNA helicase 31 (Arabidopsis thaliana)	gi 334188604	36	1%	90.03/9.02	50.26/5.1	Stress response
A44	Actin (Glycine max)	gi 18532	56	2%	41.57/5.23	44.21/5.2	Stress response
A49	Ribulose biphosphate carboxylase/oxygenase activase 2 (Nicotiana tabacum)	gi 12643758	80	11%	48.31/8.14	42.59/5.6	Photosynthesis and photorespiration
A50	Phosphoglycerate kinase (Musa acuminata)	gi 102139814	42	7%	42.27/6.20	41.78/5.7	Glycolysis and gluconeogenesis
A51	Cytosolic 3-phosphoglycerate kinase activase 2 (Nicotiana tabacum)	gi 28172913	93	18%	31.30/5.05	43.00/5.9	Glycolysis and gluconeogenesis
A52	Phosphoglycerate kinase (Ricinus communis)	gi 255544584	84	17%	50.00/8.74	43.00/6.2	Glycolysis and gluconeogenesis
A53	Allyl alcohol dehydrogenase (Nicotiana tabacum)	gi 6692816	41	5%	38.06/6.56	39.75/6.0	Fatty acid metabolism
A54	rbcL gene product (chloroplast) (Brassica napus)	gi 383930435	97	18%	52.92/5.88	42.59/6.3	Photosynthesis and photorespiration
A55	Photosystem II stability/assembly factor HCF136	gi 75252730	80	12%	45.44/9.02	38.93/5.7	Photosynthesis and photorespiration
A56	Sedoheptulose-1,7-bisphosphatase (Ricinus communis)	gi 255579134	96	5%	41.97/5.95	41.78/4.8	Photosynthesis and photorespiration
A57	Putative DNA damage repair toleration protein DRT102 (Trifolium pretense)	gi 84468444	62	3%	32.95/5.06	38.53/4.7	Stress response
A58	B3 domain-containing transcription repressor VAL2-like (Cicer arretinum)	gi 828298615	35	2%	44.49/5.75	38.53/5.0	Stress response
A59	Disease resistance protein (Theobroma cacao)	gi 16322949	42	3%	15.13/6.66	42.59/4.6	Stress response
A60	Oxygen-evolving enhancer (Pisum sativum)	gi 131384	94	10%	34.87/6.25	34.06/5.9	Photosynthesis and photorespiration
A61	Oxygen-evolving enhancer (Glycine max)	gi 356559442	105	23%	35.04/6.66	34.06/6.0	Photosynthesis and photorespiration
A64	Glyceraldehyde-3-phosphate dehydrogenase (Knorringia sibirica)	gi 115371630	90	9%	36.65/7.66	35.68/6.4	Glycolysis and gluconeogenesis
A65	Glyceraldehyde-3-phosphate dehydrogenase C subunit (Gossypium hirsutum)	gi 211906518	103	18%	36.54/7.70	39.34/6.7	Glycolysis and gluconeogenesis
A66	Glyceraldehyde-3-phosphate dehydrogenase C1 (Pyrus x bretschneideri)	gi 381393064	142	23%	36.92/8.24	39.34/6.8	Glycolysis and gluconeogenesis
A67	Putative alpha 7 proteasome subunit (Nicotiana tabacum)	gi 14594925	71	18%	27.18/6.11	30.81/6.1	Amino acid metabolism
A69	Transcription factor (Vicia faba var minor)	gi 2104681	33	2%	39.95/6.36	28.52/6.1	Cellular communication and signal transduction
A70	Triosephosphate isomerase (Coptis japonica)	gi 136057	75	8%	27.07/5.54	29.20/5.3	Glycolysis and gluconeogenesis
A71	Putative disease resistance RPP13-like protein 1 (Pyrus x bretschneideri)	gi 694327264	47	1%	200.00/5.64	30.00/5.2	Stress response
A72	Hypothetical protein SORBDRAFT_02g031030 (Sorghum bicolor)	gi 242049978	49	13%	32.34/6.45	29.43/5.2	Glycolysis and gluconeogenesis
A73	Cysteine proteinase COT44 (Brassica napus)	gi 118127	32	3%	36.25/8.05	29.43/5.0	Amino acid metabolism
A74	Oxygen-evolving enhancer protein 2, chloroplastic (Helianthus annuus)	gi 302595736	36	5%	28.12/8.67	27.85/5.0	Photosynthesis and photorespiration
A75	Oxygen-evolving enhancer protein 2 (Brugiera gymnorhiza)	gi 8131593	41	6%	17.58/4.91	27.855.2	Photosynthesis and photorespiration
A76	Heme-binding protein 2 (Cucumis sativus)	gi 449438953	57	5%	24.48/4.65	28.52/4.4	Photosynthesis and photorespiration
A77	Superoxide dismutase (Cu-Zn) (Zantedeschia aethiopica)	SODCP_ZANAE	73	12%	22.06/6.17	24.48/4.9	Stress response

(continued on next page)

Table 1 (continued)

Spot no.	Protein Identification	Accession no.	MASCOT score	% Coverage	(MW/pI) ^a Theoretical	(MW/pI) ^b Experimental	Functions
A78	Superoxide dismutase (Cu-Zn) (<i>Zantedeschia aethiops</i>)	SODCO_ZANAE	41	6%	22.06/6.17	24.48/4.8	Stress response
A79	Superoxide dismutase (Cu-Zn) (<i>Panax ginseng</i>)	SODCP_PANGI	78	8%	15.25/5.45	21.10/5.5	Stress response
A80	Maturase K (<i>Ferraria crispa</i>)	gi 71060163	31	1%	62.85/9.75	22.00/5.8	Cellular communication and signal transduction
A81	Mannose-binding lectin precursor (<i>Tulipa</i> hybrid cultivar)	gi 1141765	35	5%	18.96/4.84	26.05/6.2	Stress response
A83	Peroxidase 27 (<i>Arabidopsis thaliana</i>)	PER27_ARATH	42	3%	34.93/9.19	14.10/4.7	Stress response
A85	Probable WRKY transcription factor 43 (<i>Arabidopsis thaliana</i>)	gi 1063699318	33	9%	12.94/9.57	17.35/4.2	Stress response
A86	Mannose binding lectin AKA1 precursor (<i>Amorphophallus konjac</i>)	gi 30349401	76	7%	14.42/10.20	14.10/4.1	Stress response
Bulbophyllum morphologorum's pseudobulbs							
B3	Calcium calmodulin dependent protein kinase (<i>Medicago truncatula</i> var <i>truncatula</i>)	gi 163256950	58	7%	22.85/5.30	103.64/4.9	Stress response
B4	Nuclease HARBII (<i>Gossypium raimondii</i>)	gi 823135887	42	2%	42.00/9.70	90.35/4.6	Cellular communication and signal transduction
B5	3-ketoacyl carrier protein synthase III (<i>Allium ampeloprasum</i>)	gi 1143069	32	1%	42.62/6.40	88.14/4.7	Fatty acid metabolism
B6	Molecular chaperone hsp70b	gi 116061511	37	2%	59.76/6.60	85.92/4.9	Stress response
B7	Heat shock protein 90 (<i>Triticum aestivum</i>)	gi 294717810	300	15%	80.30/5.00	92.57/5.0	Stress response
B8	Heat shock cognate 70 kDa (<i>Vitis vinifera</i>)	gi 359486799	31	10%	71.13/5.20	83.71/5.1	Stress response
B9	Heat shock cognate 70 kDa (<i>Glycine max</i>)	gi 356568992	83	20%	71.19/5.10	81.50/5.2	Stress response
B10	High molecular weight heat shock protein (<i>Malus x domestica</i>)	gi 6969976	61	7%	71.17/5.20	81.50/5.2	Stress response
B11	P-Protein-like protein (<i>Arabidopsis thaliana</i>)	gi 14596025	34	4%	112.88/6.50	79.28/5.3	Unknown
B12	Heat shock protein 70 (<i>Phaseolus vulgaris</i>)	gi 399940	50	10%	72.49/6.00	74.85/5.4	Stress response
B13	Phosphoglycerate mutase (<i>Nicotiana attenuata</i>)	gi 111162649	59	7%	27.38/5.60	77.07/5.5	Glycolysis and gluconeogenesis
B14	2,3-bisphosphoglycerate-independent phosphoglycerate mutase (<i>Ricinus communis</i>)	PMGI_RICCO	54	3%	60.78/5.40	77.07/5.6	Glycolysis and gluconeogenesis
B15	AsnC family transcriptional regulator (<i>Propionispora</i> sp. Iso 2/2)	gi 930608178	53	8%	18.21/5.90	83.71/5.8	Cellular communication and signal transduction
B16	Chloroplast transketolase (<i>Arabidopsis lyrata</i> subsp. <i>lyrata</i>)	gi 297810173	107	5%	79.53/6.50	83.71/5.9	Stress response
B17	Hypothetical protein SELMODRAFT_403066 (<i>Selaginella moellendorffii</i>)	gi 302754452	39	2%	32.61/9.10	83.71/6.0	Unknown
B18	Methionine synthase (<i>Solanum tuberosum</i>)	gi 8439545	56	4%	84.61/5.90	88.14/6.5	Amino acid metabolism
B19	Malate dehydrogenase (<i>Cicer arietinum</i>)	gi 4586606	38	4%	17.74/5.09	74.85/6.6	Carbohydrate metabolism
B20	Histidine decarboxylase (<i>Nicotiana tomentosiformis</i>)	gi 697133277	32	1%	52.36/7.20	77.07/6.9	Amino acid metabolism
B21	Catalase 2 (<i>Elaeis guineensis</i>)	gi 192910916	67	12%	9.27/10.00	61.07/6.9	Stress response
B22	Succinate dehydrogenase (ubiquinone) flavoprotein subunit 1 (<i>Glycine max</i>)	gi 356498373	81	4%	69.72/6.30	67.10/6.3	Stress response
B23	Predicted protein (<i>Populus trichocarpa</i>)	gi 224100535	53	4%	67.54/6.60	67.10/5.7	Unknown
B24	Chaperonin CPN60 (<i>Vitis vinifera</i>)	gi 225433375	56	13%	61.33/5.90	63.53/5.4	Stress response
B25	Calreticulin-like (Phloxin dactylifera)	gi 672144143	47	3%	47.30/4.50	63.53/4.4	Stress response
B26	Enolase (<i>Elaeis guineensis</i>)	gi 353441078	90	14%	23.00/4.80	58.60/5.1	Glycolysis and gluconeogenesis
B27	Enolase (<i>Elaeis guineensis</i>)	gi 192910834	67	9%	47.73/5.98	58.60/5.2	Glycolysis and gluconeogenesis
B28	Enolase (<i>Elaeis guineensis</i>)	gi 192910834	105	11%	47.73/5.98	58.60/5.3	Glycolysis and gluconeogenesis
B29	Enolase 1 (<i>Zea mays</i>)	gi 162458207	64	4%	48.03/5.20	58.60/5.4	Glycolysis and gluconeogenesis
B30	Enolase (<i>Oryza sativa</i> Japonica Group)	gi 780372	56	8%	47.96/5.40	58.60/5.6	Glycolysis and gluconeogenesis
B31	ATP synthase CF1 alpha subunit (Phalaenopsis aphrodite subsp. <i>formosana</i>)	gi 78103238	127	12%	55.20/5.34	60.25/5.5	Photosynthesis and photorespiration
B32	S-adenosyl-L-homocysteine hydrolase (<i>Hordeum vulgare</i> subsp. <i>vulgare</i>)	gi 68655456	83	11%	49.96/5.80	59.42/5.9	Amino acid metabolism
B33	Ribulosebiphosphate carboxylase large subunit chloroplast (<i>Pogostemon cablin</i>)	gi 349048	82	9%	50.06/6.10	58.60/6.4	Photosynthesis and photorespiration
B34	Benzoate transporter (<i>Pseudomonas</i> sp. Osl17)	gi 771840651	32	2%	41.57/9.90	52.85/6.3	Cellular communication and signal transduction
B35	Unknown protein 18 (<i>Pseudotsuga menziesii</i>)	gi 205830697	78	100%	1.39/5.80	52.85/6.2	Unknown
B36	Alcohol dehydrogenase I (<i>Solanum tuberosum</i>)	gi 113365	91	12%	41.07/5.92	52.85/6.0	Stress response
B37	Peroxisomal (S)-2-hydroxy-acid oxidase 2 (<i>Aeglops tauschii</i>)	gi 475560053	24	3%	31.12/8.70	55.32/5.2	Fatty acid metabolism
B38	Elongation factor Tu (<i>Glycine max</i>)	gi 2494261	36	2%	36.35/6.20	53.67/5.4	Protein biosynthesis
B39	Predicted protein (<i>Populus trichocarpa</i>)	gi 224109060	38	4%	50.18/8.30	52.03/5.5	Cellular communication and signal transduction
B41	Cytosolic phosphoglycerate kinase (<i>Pisum sativum</i>)	gi 9230771	50	12%	42.26/5.70	52.85/5.5	Glycolysis and gluconeogenesis
B42	Actin like protein (<i>Phalaenopsis</i> sp. True Lady)	AF246715.1	56	8%	41.62/5.20	52.03/5.3	Stress response
B43	Monodehydroascorbate reductase (<i>Oncidium Gower Ramsey</i>)	gi 212896914	103	18%	46.63/5.30	52.03/5.3	Stress response

(continued on next page)

Table 1 (continued)

Spot no.	Protein Identification	Accession no.	MASCOT score	% Coverage	(MW/pI) ^a Theoretical	(MW/pI) ^b Experimental	Functions
B45	3-phosphoglycerate kinase (Hordeum vulgare subsp. vulgare)	gi 21396683	39	11%	31.32/4.90	47.921/5.2	Glycolysis and gluconeogenesis
B47	Glyceraldehyde-3-phosphate dehydrogenase (Ananas comosus)	gi 312192239	59	15%	36.57/6.70	38.47/6.9	Glycolysis and gluconeogenesis
B48	Allyl alcohol dehydrogenase (Nicotiana tabacum)	gi 6692816	30	2%	38.06/6.56	40.17/6.4	Fatty acid metabolism
B49	Plant invertase/pectin methylesterase inhibitor superfamily (Theobroma cacao)	gi 590708612	26	2%	64.48/8.10	40.73/6.0	Stress Response
B50	Hydroxyacid dehydrogenase/reductase (Medicago truncatula)	gi 124359345	47	3%	35.46/7.10	41.30/5.2	Stress response
B51	Quinone oxidoreductase (Helianthus annuus)	gi 14532287	34	2%	33.17/4.80	41.30/4.8	Stress response
B54	TMV resistance protein N-like (Nicotiana sylvestris)	gi 698528100	32	1%	129.67/7.80	39.60/5.1	Stress response
B55	la-related protein 6B-like isoform X1 (Musa acuminata subsp. malaccensis)	gi 695013984	48	2%	50.29/6.80	39.60/5.2	Cellular communication and signal transduction
B56	2-methylene-furan-3-one reductase (Solanum pennellii)	gi 970030197	81	9%	40.92/8.80	36.78/5.2	Stress response
B57	Unknown protein 18 (Pseudsuga menziesii)	gi 205830697	53	91%	1.39/5.80	35.65/5.7	Unknown
B58	Isoflavone reductase-like protein (Olea europaea)	gi 218963723	36	3%	59.54/8.70	33.39/5.8	Stress response
B59	Hypothetical protein Osl 007339 (Oryza sativa indica cultivar group)	gi 125539711	33	2%	73.55/5.50	33.39/5.5	Unknown
B60	Triosephosphate isomerase (Petunia x hybrida)	gi 1351279	70	12%	27.11/5.54	30.00/5.5	Glycolysis and gluconeogenesis
B61	Triosephosphate isomerase (Petunia x hybrida)	gi 1351279	62	10%	27.11/5.54	30.00/5.3	Glycolysis and gluconeogenesis
B62	Syntaxin-52-like (Camelina sativa)	gi 727483504	31	4%	26.07/9.07	28.11/5.6	Cellular communication and signal transduction
B63	Superoxide dismutase (Cu-Zn) (Panax ginseng)	SODCP_PANGI	34	8%	15.25/5.45	23.09/5.3	Stress response
B65	Superoxide dismutase (Cu-Zn) (Panax ginseng)	SODCP_PANGI	146	22%	15.25/5.45	21.41/5.6	Stress response
B66	Initiation factor eIF4A-15 (Helianthus annuus)	O6T8C6.HELAN	71	5%	46.58/5.30	19.88/5.8	Protein biosynthesis
B67	Intracellular pathogenesis-related protein PR-1.07 (Lilium longiflorum)	gi 4325333	59	6%	16.64/5.40	18.76/5.8	Stress response
B68	60S ribosomal export protein NMD3 (Solanum pennellii)	gi 970037034	31	1%	59.19/6.00	15.96/5.7	Stress response
B69	Glutathione-S-transferase (Avena sterilis subsp. ludoviciana)	gi 17384331	28	15%	5.79/4.70	18.76/6.4	Stress response
B70	Hypothetical protein CHLREDRAFT_173629 (Chlamydomonas reinhardtii)	gi 159472713	34	1%	94.32/6.70	18.76/6.8	Unknown
B71	Phytoene synthase (Oncidium Gower Ramsey)	gi 40557193	76	1%	46.96/7.90	15.68/6.5	Stress response
B73	Os03g0365200 (Oryza sativa japonica)	gi 115453147	59	5%	24.40/10.00	15.68/5.0	Unknown
B74	Multidrug Resistance associated Protein 1 (Catharanthus roseus)	gi 156556172	36	1%	162.66/7.50	14.84/4.8	Stress response
B75	ABC transporter C family member 9 (Glycine max)	gi 356504494	49	1%	17.01/7.33	14.84/4.6	Stress response
B76	RNA polymerase beta' subunit (Mesostigma viride)	gi 11466381	57	1%	76.73/9.15	14.84/4.4	Cellular communication and signal transduction
B77	RNA polymerase beta' subunit (Mesostigma viride)	gi 11466381	57	1%	76.73/9.15	15.68/4.0	Cellular communication and signal transduction
B79	RNA polymerase beta' subunit (Mesostigma viride)	gi 11466381	48	1%	76.73/9.15	25.81/4.6	Cellular communication and signal transduction
B80	Hypothetical protein (Oryza sativa Japonica Group)	gi 12313682	64	5%	10.78/13.00	25.81/4.8	Unknown
Dendrobium Sonia EarSakul's leaves							
C1	Pyruvate orthophosphate dikinase (Eleocharis vivipara)	gi 2285879	394	11%	95.97/5.21	98.00/5.7	Photosynthesis and photorespiration
C2	Heat shock protein 70 (Spinacia oleracea)	gi 2654208	409	18%	76.09/5.19	86.66/4.7	Stress response
C3	Heat shock protein 70 (Dendrobium catenatum)	gi 525330265	342	15%	71.46/5.13	81.50/5.2	Stress response
C4	Putative rubisco subunit binding-protein alpha subunit precursor (Oryza sativa Japonica group)	gi 31193919	139	6%	61.36/5.21	66.00/4.9	Photosynthesis and photorespiration
C5	ATP synthase beta subunit (Coriaria ruscifolia)	gi 66276267	1077	43%	50.97/5.20	60.25/5.2	Photosynthesis and photorespiration
C6	ATP synthase CF1 alpha subunit precursor (Phalaenopsis aphrodite subsp. formosana)	gi 78103238	482	22%	55.20/5.34	60.25/5.5	Photosynthesis and photorespiration
C7	Enolase (Elaeis guineensis)	gi 192910834	360	20%	47.73/5.98	57.37/5.8	Glycolysis and gluconeogenesis
C8	Enolase 1 (Guzmania wittmackii x Guzmania lingulata)	gi 365200115	425	24%	47.86/5.70	57.37/6.0	Glycolysis and gluconeogenesis
C9	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (Niedenzuella stannea)	gi 331690309	335	19%	49.40/6.19	56.41/6.4	Photosynthesis and photorespiration
C10	3-Phosphoglycerate kinase (Kengyilia hirsuta)	gi 351735496	354	35%	31.44/4.84	50.66/6.0	Glycolysis and gluconeogenesis
C11	Phosphoglycerate kinase, cytosolic (Glycine max)	gi 356525744	212	13%	42.37/6.28	46.83/5.7	Glycolysis and gluconeogenesis
C12	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic (Petunia x hybrida)	gi 120673	418	27%	36.50/6.68	40.83/6.8	Glycolysis and gluconeogenesis
C13	Probable long-chain-alcohol O-fatty-acyltransferase 3 (Brassica rapa)	gi 685282948	36	2%	39.20/9.06	37.04/5.4	Fatty acid metabolism
C14	Oxygen-evolving enhancer protein 1, chloroplastic (Vitis vinifera)	gi 147791852	200	18%	33.21/5.87	35.41/5.2	Photosynthesis and photorespiration
C15	Putative Nuclear inhibitor of protein phosphatase-1 (Zostera marina)	gi 901808822	38	1%	84.48/5.36	31.08/5.6	Fatty acid metabolism
C16	F-box family protein (Theobroma cacao)	gi 590728568	33	2%	47.82/4.60	31.08/6.1	Stress response

(continued on next page)

Table 1 (continued)

Spot no.	Protein Identification	Accession no.	MASCOT score	% Coverage	(MW/pI) ^a Theoretical	(MW/pI) ^b Experimental	Functions
C17	Carbonic anhydrase 2 (Fragment) (<i>Flaveria linearis</i>)	gi 882244	55	5%	20.57/6.21	28.20/5.8	Carbohydrate metabolism
C18	Photosystem II oxygen-evolving complex protein 2 (<i>Arabidopsis thaliana</i> (fragment))	gi 1076373	90	92%	1.43/9.71	25.50/5.8	Photosynthesis and photorespiration
C20	Early nodulin-like protein 2 (<i>Setaria italica</i>)	gi 431099	86	11%	17.66/9.39	15.59/4.2	Stress response
C21	Mannose-binding protein, partial (<i>Listera ovata</i>)	gi 835974449	49	8%	14.77/6.49	14.47/4.3	Cellular communication and signal transduction
C22	Mannose-binding protein, partial (<i>Listera ovata</i>)	gi 431099	92	11%	17.66/9.39	15.27/4.5	Stress response
C23	Lectin, partial (<i>Listera ovata</i>)	gi 431097	49	6.81%	18.65/5.52	14.10/4.8	Stress response
C24	Thioredoxin H3 (<i>Ipomoea batatas</i>)	gi 33621084	104	12%	13.70/6.06	18.13/5.0	Stress response
C25	Pyruvate orthophosphate dikinase (<i>Eleocharis vivipara</i>)	gi 2285879	269	12%	95.97/5.21	98.00/5.7	Photosynthesis and photorespiration
C26	UDP-glucose-1-phosphate uridylyltransferase (<i>Hordeum vulgare</i>)	gi 6136111	63	2%	51.78/5.20	53.54/5.1	Stress response
C27	Ribulose-1,5-bisphosphate carboxylase/oxgenase (<i>Haemophilia vitata</i>)	gi 33635955	197	14%	49.17/6.43	57.37/6.2	Photosynthesis and photorespiration
C28	ATP synthase subunit beta-3 (<i>Arabidopsis thaliana</i>)	gi 22326673	415	21%	59.82/6.06	57.37/5.3	Photosynthesis and photorespiration
C29	Sedoheptulose-1,7-bisphosphatase, chloroplast putative (<i>Ricinus communis</i>)	gi 255579134	148	9%	41.97/5.95	43.95/4.9	Carbohydrate metabolism
C30	Phosphoribulokinase (<i>Spinacia oleracea</i>)	gi 125579	72	9%	44.98/5.82	43.00/5.2	Stress response
C31	Actin (<i>Gossypium hirsutum</i>)	gi 32186894	249	26%	41.67/5.31	49.70/5.4	Stress response
C32	Ribulose biphosphatase carboxylase/oxgenase activase (<i>Solanum pennellii</i>)	gi 10720247	59	13%	50.67/8.61	44.92/5.5	Photosynthesis and photorespiration
C33	Monodehydroascorbate reductase (<i>Malus x domestica</i>)	gi 225380882	53	3%	46.88/6.51	48.75/6.2	Stress response
C34	Fructose-bisphosphate aldolase (<i>Codonopsis lanceolata</i>)	gi 82941449	62	7%	38.14/6.47	41.37/6.4	Glycolysis and gluconeogenesis
C35	NAD-dependent malate dehydrogenase (<i>Prunus persica</i>)	gi 15982948	41	7%	35.82/6.60	39.21/6.2	Carbohydrate metabolism
C36	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic (<i>Petunia x hybrida</i>)	gi 120673	362	22%	36.50/6.68	38.66/6.5	Glycolysis and gluconeogenesis
C37	Probable adenylate kinase 6 (<i>Tarenaya hassleriana</i>)	gi 729401807	46	3%	33.44/6.26	37.58/6.1	Amino acid metabolism
C38	Probable disease resistance protein RXW241 (<i>Arabidopsis thaliana</i>)	gi 6566297	35	1%	104.27/6.62	34.87/6.3	Stress response
C39	Triosephosphate isomerase (<i>Coptis japonica</i>)	gi 136057	59	5%	27.07/5.54	31.35/5.2	Glycolysis and gluconeogenesis
C40	Triosephosphate isomerase (<i>Coptis japonica</i>)	gi 136057	85	8%	27.07/5.54	29.55/5.6	Glycolysis and gluconeogenesis
C41	Putative cytochrome c oxidase subunit II PS17 (<i>Pinus strobus</i>)	gi 109892850	26	50%	1.71/9.62	25.05/5.1	Photosynthesis and photorespiration
C42	Carbonic anhydrase (<i>Arabidopsis thaliana</i>)	gi 15220853	65	4%	28.81/6.59	28.20/6.2	Carbohydrate metabolism
C43	Probable adenylate kinase 6 (<i>Tarenaya hassleriana</i>)	gi 729401807	42	3%	33.44/6.26	27.07/6.4	Amino acid metabolism
C44	PsbP domain-containing protein 4, chloroplastic (<i>Arabidopsis thaliana</i>)	gi 2829916	49	6%	28.48/7.02	24.93/6.2	Photosynthesis and photorespiration
Dendrobium. Sonia Earsakul's pseudobulbus							
D2	Hypothetical protein CISIN_1g037404mg (<i>Citrus sinensis</i>)	gi 641853885	68	10%	68.46/8.19	81.50/5.2	Unknown
D3	Phosphoglycerate mutase (<i>Arabidopsis thaliana</i>)	gi 2160168	67	2%	62.63/5.36	79.28/5.4	Glycolysis and gluconeogenesis
D4	Heat shock protein 70 (<i>Capsicum annuum</i>)	gi 163311872	153	16%	7.40/4.76	70.42/5.8	Stress response
D5	NADP-dependent malic enzyme 1 (<i>Arabidopsis thaliana</i>)	gi 15225262	41	6%	64.24/6.32	70.42/5.9	Glycolysis and gluconeogenesis
D6	Phosphoribulokinase (<i>Monoraphidium neglectum</i>)	gi 926792189	36	5%	26.15/8.91	574.85/7.9	Stress response
D7	Hsp70-Hsp 90 organizing protein 2 (<i>Arabidopsis thaliana</i>)	gi 58331773	46	2%	64.48/5.85	79.28/5.9	Stress response
D8	Malate dehydrogenase (<i>Citrus arifetinum</i>)	gi 4586606	86	4%	17.74/5.09	66.00/6.8	Carbohydrate metabolism
D9	NADP-dependent malic enzyme 1 (<i>Arabidopsis thaliana</i>)	gi 15225262	41	6%	64.24/6.32	62.32/6.2	Glycolysis and gluconeogenesis
D10	Aldehyde dehydrogenase family 2 member B7, mitochondrial (<i>Morus notabilis</i>)	gi 21410404	84	2%	58.01/6.16	58.64/6.3	Stress response
D11	Aldehyde dehydrogenase family 2 member (<i>Morus notabilis</i>)	gi 703113828	85	2%	58.40/6.16	59.56/6.2	Stress response
D12	F1-ATPase alpha subunit (<i>Calamus usitatus</i>)	gi 1381685	38	5%	45.79/7.89	59.56/5.9	Photosynthesis and photorespiration
D13	D-3-phosphoglycerate dehydrogenase (<i>Phoenix dactylifera</i>)	gi 672132227	53	4%	66.01/6.36	59.56/5.8	Fatty acid metabolism
D14	ATP synthase subunit alpha (<i>Phalaenopsis aphrodite</i> subsp. formosana)	gi 78103238	162	14%	55.20/5.43	59.56/5.7	Photosynthesis and photorespiration
D15	Enolase 1 (<i>Zea mays</i>)	gi 162458207	64	3%	48.26/5.20	59.56/5.4	Glycolysis and gluconeogenesis
D16	Phosphoribulokinase (<i>Monoraphidium neglectum</i>)	gi 926792189	36	5%	26.15/8.91	61.40/5.4	Stress Response
D17	Mitochondrial F1 ATP synthase beta subunit (<i>Arabidopsis thaliana</i>)	gi 17939849	200	17%	63.33/6.52	64.62/5.4	Photosynthesis and photorespiration
D18	Enolase 2 (<i>Hevea brasiliensis</i>)	gi 14423687	73	100%	48.11/5.92	60.48/5.2	Glycolysis and gluconeogenesis
D19	ABC transporter C family member 9 (<i>Glycine max</i>)	gi 356504494	46	1%	17.01/7.33	61.40/5.1	Stress response
D20	D-3-phosphoglycerate dehydrogenase (<i>Phoenix dactylifera</i>)	gi 672132227	53	4%	66.01/6.36	52.20/5.4	Fatty acid metabolism
D21	Actin-3 (<i>Oryza sativa</i> subsp. Indica)	gi 20331	70	15%	41.68/5.31	51.28/5.7	Stress response
D22	ATP synthase CF1 alpha subunit (<i>Phalaenopsis aphrodite</i> subsp. formosana)	gi 78103238	127	12%	55.20/5.34	53.12/5.8	Photosynthesis and photorespiration
D23	Unknown protein 18 (<i>Vitis rotundifolia</i>)	gi 205830697	78	100%	1.39/5.80	51.28/6.1	Unknown
D24	ATPase alpha subunit (<i>Thalassia testudinum</i>)	gi 114509234	165	9%	13.53/5.61	53.12/6.3	Photosynthesis and photorespiration
D25	Glyceraldehyde-3-phosphate dehydrogenase (<i>Xerocladia viridiramis</i>)	gi 158421228	243	29%	5.08/10.20	43.92/6.3	Glycolysis and gluconeogenesis
D26	Alcohol dehydrogenase 1 (<i>Solanum tuberosum</i>)	gi 113365	91	12%	41.07/5.92	43.00/6.4	Stress response

(continued on next page)

Table 1 (continued)

Spot no.	Protein Identification	Accession no.	MASCOT score	% Coverage	(MW/pI) ^a Theoretical	(MW/pI) ^b Experimental	Functions
D27	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic (Craterostigma plantagineum)	gi 460979	38	11%	36.45/7.06	42.35/6.5	Glycolysis and gluconeogenesis
D28	Cytochrome c reductase 53 kDa subunit P1 peptide	gi 633898	231	31%	0.21/9.87	43.00/6.7	Photosynthesis and photorespiration
D29	Glyceraldehyde-3-phosphate dehydrogenase (Mallotus nesophilus)	gi 156617106	83	12%	6.50/10.70	41.70/6.8	Glycolysis and gluconeogenesis
D30	2-alkenal reductase (NADP(+)-dependent) (Nicotiana tabacum)	gi 444302249	56	10%	38.06/6.56	35.20/6.5	Unknown
D33	MORC family CW-type zinc finger 3a (Zostera marina)	gi 901809830	39	1%	66.92/6.05	39.36/6.0	Fatty acid metabolism
D34	Glyoxalase I homologue 1 (Allium cepa)	gi 332629595	61	14%	33.32/5.88	43.00/5.5	Stress response
D35	20 kDa chaperonin, chloroplastic-like (Oryza brachyantha)	gi 573923036	41	3%	38.64/5.88	39.75/5.2	Glycolysis and gluconeogenesis
D36	Triosephosphate isomerase TPI (Lactuca sativa)	gi 256124	212	27%	4.67/4.43	36.50/5.2	Glycolysis and gluconeogenesis
D37	Serine/threonine-protein kinase (Vitis vinifera)	gi 225462205	34	2%	43.06/6.35	35.85/4.8	Fatty acid metabolism
D38	Quinone oxidoreductase like protein (<i>Arabidopsis thaliana</i>)	gi 21553644	87	8%	32.71/5.78	31.95/5.3	Photosynthesis and photorespiration
D39	Oxygen-evolving enhancer protein 1, chloroplastic (Fritillaria agrestis)	gi 11133881	77	10%	34.85/6.26	30.00/5.4	Photosynthesis and photorespiration
D40	2-methylene-furan-3-one reductase (Solanum lycopersicum)	gi 743758187	273	13%	41.85/8.97	29.34/5.5	Stress response
D41	2-methylene-furan-3-one reductase (Solanum lycopersicum)	gi 743758187	30	6%	40.98/7.74	31.30/5.6	Stress response
D42	Chloroplast photosynthetic water oxidation complex 33 kDa subunit precursor (Morus nigra)	gi 152143640	121	10%	28.25/5.30	24.95/5.6	Photosynthesis and photorespiration
D43	Triosephosphate isomerase (Zea mays)	gi 195605636	174	16%	27.28/5.53	29.34/5.8	Glycolysis and gluconeogenesis
D44	Nuclear inhibitor of protein phosphatase-1 (Zostera marina)	gi 901808822	34	1%	84.48/5.36	36.50/6.0	Fatty acid metabolism
D45	Triosephosphate isomerase (Petunia x hybrid)	gi 1351279	99	12%	27.11/5.54	33.12/6.1	Glycolysis and gluconeogenesis
D47	Proteasome subunit alpha type-3 (<i>Arabidopsis thaliana</i>)	gi 51970040	30	11%	27.36/5.93	32.60/6.4	Amino acid metabolism
D48	Glyceraldehyde-3-phosphate dehydrogenase C1 (Pyrus x bretschneideri)	gi 381393064	64	9%	36.92/8.24	29.12/6.5	Glycolysis and gluconeogenesis
D49	Glyceraldehyde-3-phosphate dehydrogenase (Xerocladia viridiramis)	gi 158421228	187	9%	5.08/10.20	29.34/6.9	Glycolysis and gluconeogenesis
D50	Glyceraldehyde-3-phosphate dehydrogenase (Lilium longiflorum)	gi 83839213	87	8%	35.06/6.43	26.70/6.8	Glycolysis and gluconeogenesis
D51	Monodehydroascorbate reductase (Acanthos ebracteatus)	gi 117067068	96	10%	46.55/5.15	26.92/6.3	Stress response
D52	Triosephosphate isomerase (Zea mays)	TPIS_MAIZE	132	13%	27.01/5.37	27.58/6.0	Glycolysis and gluconeogenesis
D53	Syntaxin-52-like (Camelina sativa)	gi 727483504	31	4%	26.07/9.07	26.92/5.8	Cellular communication and signal transduction
D54	Adenylate kinase 6 (Tarenaya hassleriana)	gi 729401807	40	3%	33.44/6.26	25.61/5.7	Amino acid metabolism
D55	Triosephosphate isomerase (Fragaria vesca subsp. vesca)	gi 470143704	214	16%	27.40/6.34	25.17/5.5	Glycolysis and gluconeogenesis
D56	LRR repeats and ubiquitin-like (Pyrus x bretschneideri)	gi 694387665	45	10%	14.66/6.82	26.27/5.5	Stress response
D57	Cytokinesis related Sec1 protein like (Oryza sativa Japonica Group)	gi 47497438	77	5%	27.33/5.45	27.15/4.9	Cellular communication and signal transduction
D58	Predicted protein (Physcomitrella patens subsp patens)	gi 168062920	56	1%	170.77/6.11	21.65/5.3	Unknown
D59	Phosphoinositide 4-kinase (Theobroma cacao)	gi 590679345	36	1%	66.09/5.85	22.31/5.5	Stress response
D61	BnaC07g10230D (Brassica napus)	gi 674938758	40	2%	4.29/4.66	22.31/5.7	Unknown
D62	Maturase K (Parkinsonia aculeate)	gi 68052508	57	1%	60.21/9.30	22.31/5.9	Cellular communication and signal transduction
D63	Pathogenesis related protein (Asparagus officinalis)	gi 510940	51	6%	16.47/7.19	18.20/6.7	Stress response
D64	LRR receptor-like serine/threonine-protein kinase GSO2 (Aegilops tauschii)	gi 475555744	35	10%	131.06/6.21	17.92/6.8	Stress response
D65	Phosphoethanolamine N-methyltransferase 1 (Cucumis sativus)	gi 449439453	36	2%	57.15/5.35	14.56/6.9	Fatty acid metabolism
D66	Mediator of RNA polymerase II transcription subunit 17 (Jatropha curcas)	gi 802640310	31	1%	74.57/5.74	14.56/6.2	Photosynthesis and photorespiration
D67	ABC transporter C family member 9 (Glycine max)	gi 356504494	46	1%	17.01/7.33	15.40/5.5	Cellular communication and signal transduction
D68	Dihydroflavonol 4-reductase (Rosa hybrid cultivar)	gi 1332411	61	3%	39.00/5.94	17.92/5.2	Stress response
D69	DNA-directed RNA polymerase subunit beta' (Mesostigma viride)	gi 13878754	42	1%	76.73/9.15	14.84/4.6	Cellular communication and signal transduction
D70	RNA polymerase beta' subunit (Mesostigma viride)	gi 11466381	57	1%	76.73/9.15	14.84/4.2	Cellular communication and signal transduction

Note: All spots in this table are statistically significant at $p < 0.05$. ^a Theoretical molecular weight and pI were from MASCOT database, ^b Experimental molecular weight and pI were from our gels.

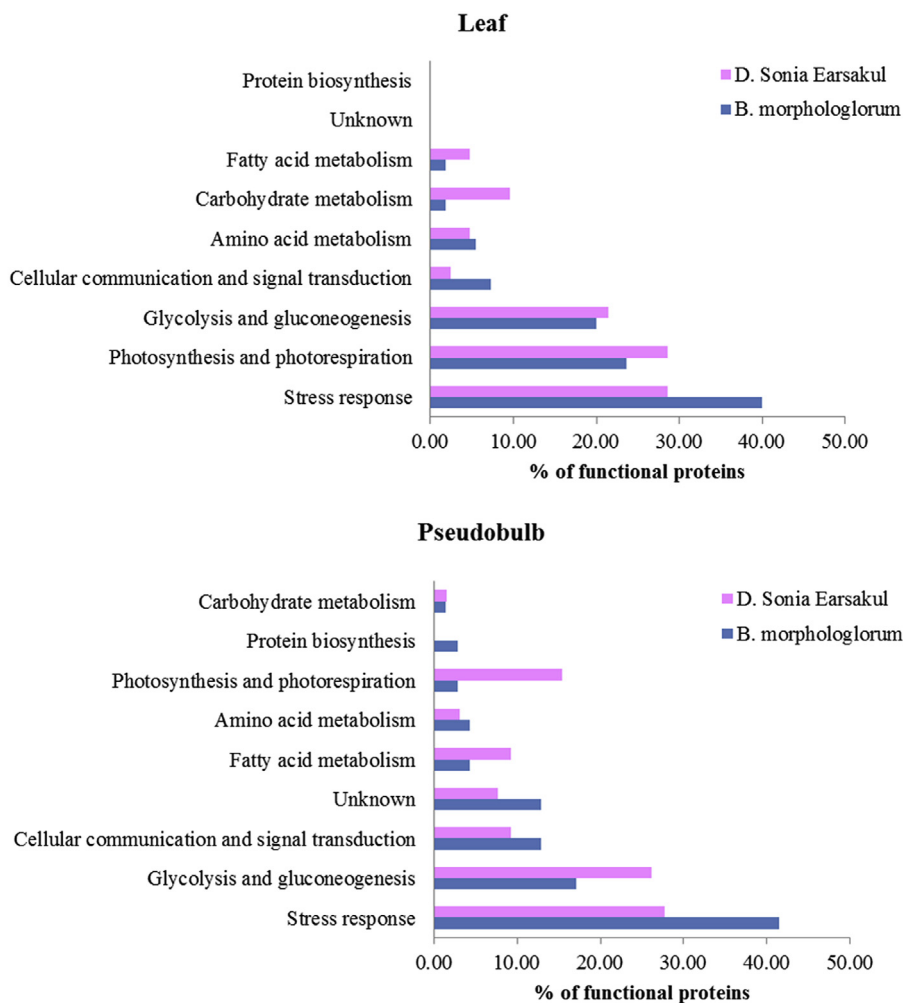


Fig. 2. Functional annotation of highly expressed proteins from leaves and pseudobulbs of *Bulbophyllum morphologlorum* Kraenzl. and *Dendrobium* Sonia Earsakul are shown as bar graphs.

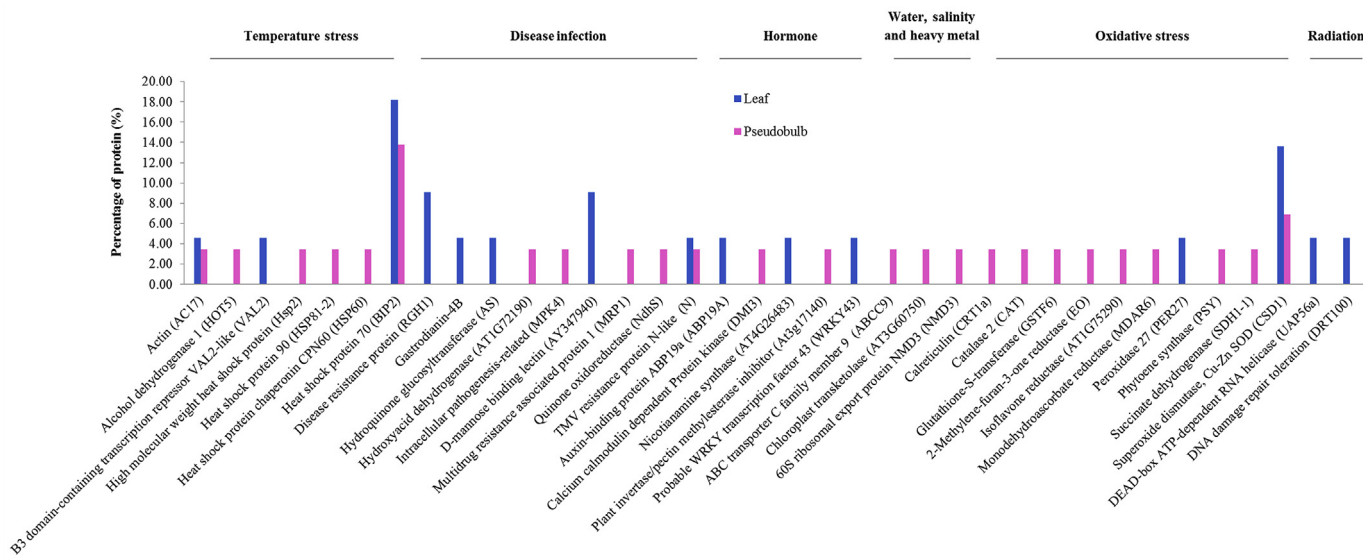


Fig. 3. Percentage of the stress proteins associated with biotic stress (infection) and abiotic stress (temperature, hormone, water, salt, metal) in orchid leaves and pseudobulbs of *Bulbophyllum morphologlorum* Kraenzl.

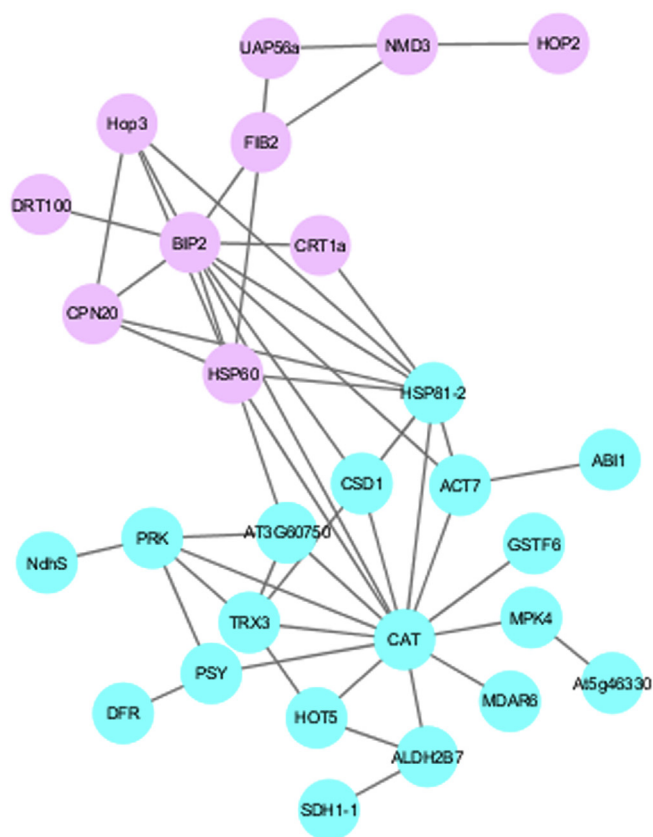


Fig. 4. The interaction network of proteins involved in stress response of leaves and pseudobulbs of *Bulbophyllum morphologlorum* Kraenzl. The 2 major clusters are shown in pink and blue, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

orchids after ammonium sulfate precipitation were added with non-reducing sample buffer (62.5 mM Tris-HCl pH 6.8, 10%, v/v glycerol and 1%, w/v bromophenol blue) and loaded onto native-PAGE. Electrophoresis was performed for 60 min at 4 °C and 10 mA. SOD activity was detected by incubating the gel in staining buffer (50 mM phosphate, pH 7.8), containing EDTA (1 mM) and riboflavin-NBT in the dark for 10 min. The riboflavin-NBT was replaced by 0.1% v/v TEMED and left in the dark for 15 min. Then the solution was removed and the gel was placed under a 25 W light bulb until SOD bands were visualized. The SOD bands were confirmed by in-gel tryptic digestion and LC/MS/MS using the above method.

3. Results

3.1. Protein profiles of leaves and pseudobulbs of *Bulbophyllum morphologlorum* Kraenzl and *Dendrobium Sonia* Earsakul

Three hundred micrograms of phenol extracted proteins from leaves and pseudobulb of *Bulbophyllum morphologlorum* Kraenzl and *Dendrobium Sonia* Earsakul were separately loaded in triplicate onto 2-DE gels. The results showed reproducible and clear proteomic maps with distinctive and intense spots ranging from 14 to 97 kDa as shown in Fig. 1 (A-D). ImageMaster 2D Platinum software was used for analysis, showing that the *Bulbophyllum* leaf and pseudobulb extracts had 700 and 673 protein spots, respectively while the *Dendrobium* leaf and pseudobulb extracts had 679 and 551 protein spots, respectively. A total of 233 randomly selected protein spots of highly expressed proteins from both tissues of *Bulbophyllum* and *Dendrobium* were excised and trypsinized for identification of proteins by LC-MS/MS analysis.

3.2. Protein identification by LC-MS/MS analysis

The highly expressed protein spots of interest, selected as representative proteins from the leaves and pseudobulbs of the *Dendrobium* and *Bulbophyllum*, were digested with trypsin and identified by LC-MS/MS. A total of 233 proteins were identified using SWISSPROT databases as annotated proteins (Table 1) including accession number, Mascot score, percent coverage, MW/pI (experimental and theoretical) and functions, using the criteria explained in the Materials and Methods. Since there is still no database for orchids, we searched by using viridiplantae (green plants) from the database. The identified proteins were from various types of plants that matched with the peptide sequences. Based on the Protein Analysis Through Evolutionary Relationships (PANTHER) Gene Ontology classification analyses, these 233 annotated proteins were categorized and displayed by the percent of proteins into 9 functional groups as follows: proteins involved in amino acid metabolism, carbohydrate metabolism, cellular communication and signal transduction, fatty acid metabolism, glycolysis and gluconeogenesis, photosynthesis and photorespiration, protein biosynthesis, stress response and unknown proteins. The functional proteins in the leaves of the *Bulbophyllum* were annotated into stress response (40%), photosynthesis and photorespiration (23.64%), and glycolysis and gluconeogenesis group (20%). In comparison, the proteins in pseudobulbs of the *Bulbophyllum* were dominated by stress response (41.43%), glycolysis and gluconeogenesis (17.14%), and cellular communication and signal transduction (12.86%) (Fig. 2).

The thirty-six differentially expressed proteins from leaves and pseudobulbs of *Bulbophyllum* were mainly involved in stress activities and defense mechanisms and were classified into six sub-groups based on their role in responding to stress conditions as shown in Fig. 3, including temperature stress, disease infection, hormone, water, salinity and heavy metal, oxidative stress (enzymatic and non-enzymatic) and radiation. The stress response proteins associated with temperature stress and oxidative stress were most involved with heat shock protein 70 and superoxide dismutase (Cu-Zn), respectively. The gene names are also shown in addition to the protein names.

3.3. Protein-protein interaction network of stress response proteins from *Bulbophyllum morphologlorum* Kraenzl

The interaction network with the confidence score for the 36 stress response proteins from *Bulbophyllum* orchids was obtained by using the STRING database. The STRING was able to help predict the related functions of proteins obtained by accessing many free databases. Visualization of the network was performed by Cytoscape software. The clustering of biological processes was represented by different colors according to the related functions. The results for the network interaction (Fig. 4) indicate two clusters of high expression proteins, including proteins involved in response to temperature stress (ACT7, HOTS, CPN20, HSP81-2, HSP60, HOP2, HOP3 AND BIP2) and in oxidative stress (ALDH2B7, CRT1A, CAT, DFR, GSTF6, MDAR6, PSY, SDH1-1, CSD1 AND TRX3), respectively. Heat shock protein 70 (BIP2) and catalase 2 (CAT) were 2 proteins that showed core interaction with other proteins.

3.4. Validation of superoxide dismutase (Cu-Zn) by Native-PAGE and confirmed by LC/MS/MS

Extracted proteins from orchid, after ammonium sulfate precipitation, were subjected to native-PAGE, and incubated in riboflavin-NBT solution and treated with 25 W light exposure to induce superoxide synthesis. Six bands (band I, II, III, IV, V and VI) of superoxide dismutase activity were obtained from leaves (BML) and pseudobulbs (BMP) of *Bulbophyllum* orchid (Fig. 5A), All six bands were cut, digested by trypsin and analyzed by LC/MS/MS. Based on SWISSPROT database, Cu/Zn-SOD isoenzymes were only identified in band IV and VI as Cu/

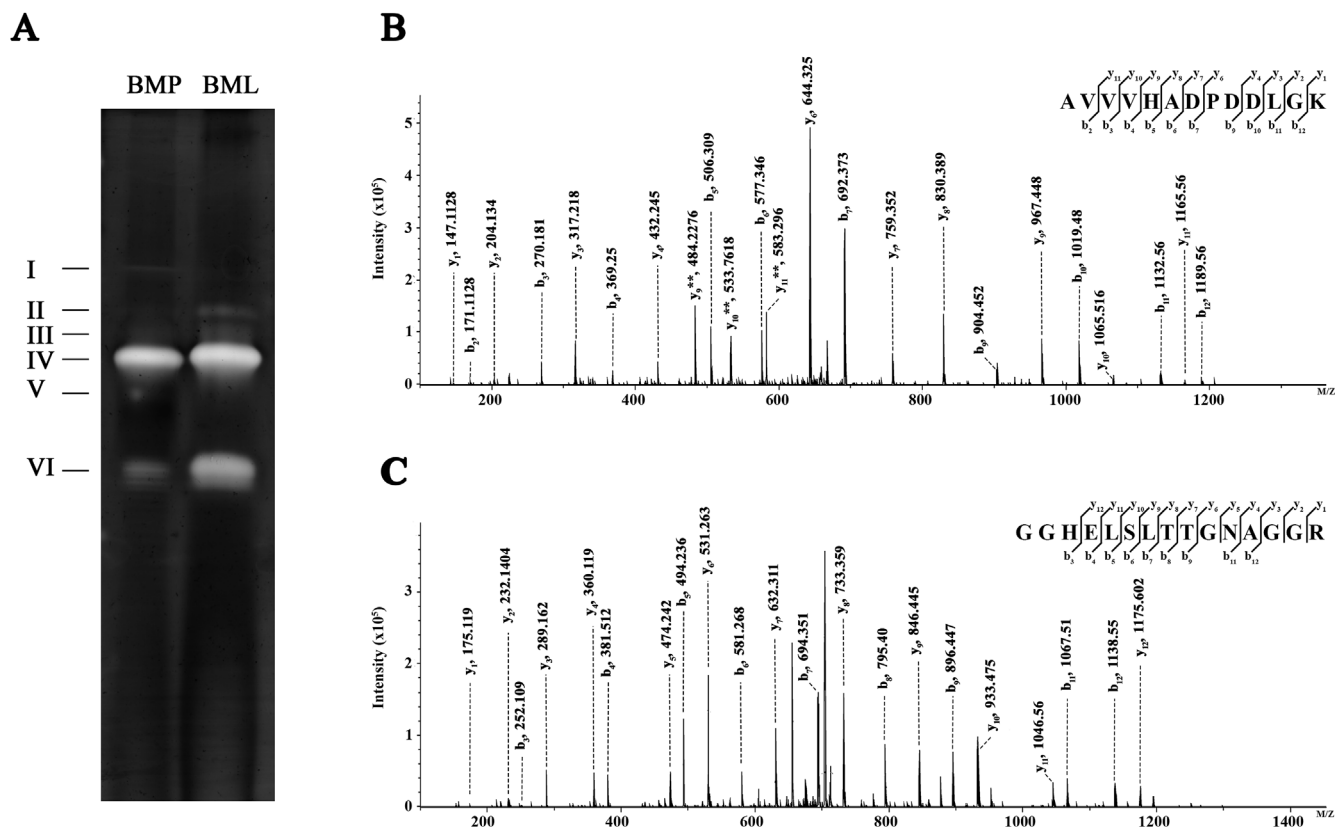


Fig. 5. The Native PAGE of Superoxide dismutase isoenzyme activities in leaves and pseudobulbs of *Bulbophyllum morphologlorum* Kraenzl were shown (A). Representative MS/MS spectra of identified peptides from band IV (B) and VI (C) were AVVVHADPDDLGGK of Cu/Zn-SOD 1 and GGHELSTLTGNAGGR of Cu/Zn-SOD 2, respectively.

Table 2
Identification of protein bands (I-VI) from SOD activity native gels.

Gel band	Identified protein (species)	Accession no.	Score	Peptide match	Unique seq.	pI/MW	Peptides
I	Enolase 1 (<i>Zea mays</i>)	ENO1_MAIZE	548	1	1	5.20/48.03	R.IEEELGDAAVYAGAK.F
II	Enolase 1 (<i>Zea mays</i>)	ENO1_MAIZE	462	6	1	5.20/48.03	K.IPLYQHIANLAGNK.T K.EGLELLK.A K.TCNALLLK.V K.YNQLLR.I R.IEEELGDAAVYAGAK.F K.FRAPPVEPY
III	Enolase 1 (<i>Zea mays</i>)	ENO1_MAIZE	1542	8	1	5.20/48.03	K.KIPLYQHIANLAGNK.T K.IPLYQHIANLAGNK.T K.EGLELLK.A K.DKTYDLNFK.E K.TCNALLLK.V K.YNQLLR.I R.IEEELGDAAVYAGAK.F K.FRAPPVEPY
IV	Superoxide dismutase [Cu-Zn] 1 (<i>Arabidopsis thaliana</i>)	SODC1_ARATH	126	2	2	5.54/15.25	QIPLIGSGSIIGR.A R.AVVVHADPDDLGGK.G
V	Enolase 1 (<i>Zea mays</i>)	ENO1_MAIZE	136	3	1	5.20/48.03	K.TCNALLLK.V K.YNQLLR.I
VI	Superoxide dismutase [Cu-Zn] 2 (<i>Arabidopsis thaliana</i>)	SODC2_ARATH	170	2	2	6.48/22.23	R.IEEELGDAAVYAGAK.F R.AFVVHVELKDDLGGK.G K.GGHELSTLTGNAGGR.L

Zn-SOD 1 and Cu/Zn-SOD 2, respectively (Table 2). There were no significant differences in the activity of Cu/Zn-SOD 1 between BML and BMP. In contrast, the elevated Cu/Zn-SOD 2 activity was obviously detected in BML as compared to BMP. Representative MS/MS spectra of the sequence specific peptides for Cu/Zn-SOD 1 and Cu/Zn-SOD 2 were shown as AVVVHADPDDLGGK and GGHELSTLTGNAGGR, respectively (Fig. 5B and C).

4. Discussion

Antioxidant defenses are used to neutralize reactive oxygen and nitrogen species (RONS) which occur from both endogenous and exogenous processes to produce negative effects. When there is an imbalance between RONS and antioxidant defenses, oxidative stress occurs. During aging, the organ and tissue functions are progressively lost and involve oxidative stress related to many diseases such as

cardiovascular disease, cancer, chronic kidney disease, neurodegenerative disease and etc [53] Natural antioxidants from plants have received much attention and have proven to be useful for preventing related oxidative stress diseases, thereby slowing ageing processes. Our results showed the *Bulbophyllum* ethanol crude extract had stronger exogenous antioxidant activities against free radical molecules than other orchid extracts. Usually, tolerant plants are reported to contain high antioxidants in order to protect from oxidative stress and keep maintaining a high amount under stress conditions.

The differential protein expression of phenol extracted proteins from leaves and pseudobulbs of *Bulbophyllum morphologorum* Kraenzl. and *Dendrobium Sonia* Earsakul were compared by proteomic methods. A total of 233 proteins from selected spots were identified from *Bulbophyllum* and *Dendrobium* leaves and pseudobulbs. The predominant protein groups found in both orchids, particularly proteins in leaves and pseudobulbs of *Bulbophyllum* orchid, were involved in stress response. Interestingly, more than half of the annotated stress proteins highly expressed in *Bulbophyllum* were associated with temperature stress and oxidative stress response function. The protein-protein interaction network also showed clusters of antioxidant defense and heat shock proteins, respectively. Proteins from both leaves and pseudobulbs of *Bulbophyllum* that are involved in temperature stress are actin, alcohol dehydrogenase 1, B3 domain-containing transcription repressor, high molecular weight heat shock protein, heat shock protein 90, heat shock protein chaperonin CPN60 and heat shock protein 70 (HSP70). The most abundant protein identified in pseudobulbs of *Bulbophyllum* was HSP70. HSP70 proteins from leaf tissue play essential roles in various mechanisms, such as refolding protein conformations and protecting against harmful effects of abiotic stress [54,55]. Generally, a number of plant HSPs were detected in leaf and green tissues [56]. However, the expression of HSP70 was shown to be up-regulated in the mycorrhizal *Bipinnula fimbriata* roots cultured in heavy metal-polluted soil [43]. In addition, HSP90 has been reported to act as a co-chaperone, forming a chaperone complex with HSP70, which regulates a resistance gene in wheat [57] and *Arabidopsis* [58].

Proteins highly involved in oxidative stress response include calreticulin, catalase 2, glutathione-S-transferase, 2-methylene-furan-3-one reductase, isoflavone reductase, monodehydroascorbate reductase, peroxidase 27, phytoene synthase, succinate dehydrogenase and superoxide dismutase (Cu-Zn). The expression of enzymatic antioxidants from our work includes catalase 2, glutathione-S-transferase and Cu/Zn-SOD. One of the most important enzymatic antioxidants is SOD which showed high expression in both leaves and pseudobulbs of *Bulbophyllum* orchids, also detected by SOD activity staining on native-PAGE. LC/MS/MS was used to identify the type of SOD isoenzymes from activity bands, confirming the presence of Cu/Zn-SOD 1 and Cu/Zn-SOD 2. This is the first report on the Cu/Zn-SOD in the *Bulbophyllum* orchids. Our finding suggests that Cu/Zn-SOD 2 activity was highly elevated on *Bulbophyllum* leaves, as compared to *Bulbophyllum* pseudobulbs, whereas there were no differences in Cu/Zn-SOD 1 activity. In agreement with previous studies [59], Cu/Zn-SOD 2 is mainly localized in the plant chloroplast.

Antioxidants from natural sources have been shown to be good potential medicines for maintaining health, preventing oxidative stress related diseases and delaying the process of aging [60]. Antioxidants may also be used in cosmetics and food supplements [[61]]. Potato, legumes, berries, spinach, tomatoes, cherries, prunes, olives and citrus were identified to be non-enzymatic antioxidant sources [62,63], as well as some orchids [64]. Studies on searching for new and safe endogenous antioxidants, of both enzymatic and non-enzymatic nature, from natural sources, is still of interest for use as supplements for antioxidant defense to prevent and manage oxidative stress related diseases. Our results suggest that *Bulbophyllum* orchid has the higher activity of Cu/Zn-SOD than of *Dendrobium* and can be a potential plant source for medicines and natural antioxidant supplements.

5. Conclusions

Proteomic study of the phenol extracted proteins of *Bulbophyllum* and *Dendrobium* led to distinctive and intense protein spots on 2-DE gel, allowing 233 proteins to be identified using LC-MS/MS analysis. Search for protein functions showed that the predominant annotated proteins in both orchids were stress response proteins, mostly associated with antioxidant and temperature which showed more variability in the *Bulbophyllum* than *Dendrobium*. Proteins related to stress conditions, such as heat shock proteins and Cu/Zn-SOD, showed particularly high expression in *Bulbophyllum*. The high expression of this antioxidant enzyme from *Bulbophyllum morphologorum* Kraenzl was confirmed using superoxide dismutase activity staining on native-PAGE coupled with LC/MS/MS. The activity of Cu/Zn-SOD 2 was highly elevated on *Bulbophyllum* leaves as compared to *Bulbophyllum* pseudobulbs whereas there were no differences in Cu/Zn-SOD 1 activity. The results suggest that *Bulbophyllum* orchid can be a potential plant source for medicines and natural antioxidant supplements.

Author contributions

KB conducted the experiments. JS, CS and DC provided analytical tools and supervised 2DE and Image Master analysis. DC and CS identified proteins using LC-MS/MS. CW analyzed data using STRING, Cytoscape software and part of the mass spectrometry. PSH and SM conceived and designed experiment. PSH and CS analyzed data and wrote the manuscript. JS read and corrected the manuscript. All authors read and approved the final manuscript.

Ethical standards

Compliance with ethical standards.

Declaration of competing interest

The authors declared that they have no conflict of interest.

Acknowledgements

This work was supported by grants from the Chulabhorn Research Institute grant (BT 2011-01). The authors thank the Laboratory of Biotechnology and Laboratory of Biochemistry, Chulabhorn Research Institute for providing facilities.

References

- [1] E. Sikora, E. Cieřlik, T. Leszczynęska, A. Filipiak-Floriewicz, P.M. Pisulewski, The antioxidant activity of selected cruciferous vegetables subjected to aquathermal processing, *Food Chem.* 107 (1) (2008) 55–59.
- [2] M.S. Brewer, Natural antioxidants: sources, compounds, mechanisms of action, and potential applications, *Compr. Rev. Food Sci. Food Saf.* 10 (4) (2011) 221–247.
- [3] T. Beta, S. Nam, J.E. Dexter, H.D. Sapirstein, Phenolic content and antioxidant activity of pearled whet and roller-milled fractions, *LWT- Food Sci. Tech.* 78 (2017) 151–159.
- [4] X. Chen, S. Liu, P. Rao, J. Bradshaw, R. Weller, Topical application of superoxide dismutase mediated by HIV-TAT peptide attenuates UVB-induced damages in human skin, *Eur. J. Pharm. Biopharm.* 107 (2016) 286–294.
- [5] L. Papa, G. Manfredi, D. Germain, SOD1, an unexpected novel target for cancer therapy, *Genes & Cancer* 5 (1–2) (2014) 15–21.
- [6] O.M. Ighodaro, O.A. Akinloye, First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defense grid, *Alexandria J. Med.* 54 (2018) 287–293.
- [7] S.M. Bridges, M.L. Salin, Distribution of iron-containing superoxide dismutase in vascular plants, *Plant Physiol.* 68 (2) (1981) 275–278.
- [8] C. Bowler, C.W. Van, M.M. Van, D. Izne, K. Asada, Superoxide dismutase in plants, *Crit. Rev. Plant Sci.* 13 (3) (1994) 199–218.
- [9] R.G. Alscher, N. Erturk, L.S. Heath, Role of superoxide dismutases (SODs) in controlling oxidative stress in plants, *J. Exp. Bot.* 53 (372) (2002) 1331–1341.
- [10] F.J. Corpas, A. Fernández-Ocaña, A. Carreras, R. Valderrama, F. Luque, F.J. Esteban, M. Rodríguez-Serrano, M. Chaki, J.R. Pedrajas, L.M. Sandalio, L.A. del Río, J.B. Barroso, *Plant Cell Physiol.* 47 (7) (2006) 984–994.

- [11] D.J. Kliebenstein, R.A. Monde, R.L. Last, Superoxide dismutase in Arabidopsis: an eclectic enzyme family with disparate regulation and protein localization, *Plant Physiol.* 118 (2) (1998) 637–650.
- [12] K.H. Baek, D.Z. Skinner, Alteration of antioxidant enzyme gene expression during cold acclimation of near isogenic wheat lines, *Plant Sci.* 165 (2003) 1221–1227, [https://doi.org/10.1016/S0168-9452\(03\)00329-7](https://doi.org/10.1016/S0168-9452(03)00329-7).
- [13] J.F. Moran, E.K. James, M.C. Rubio, G. Sarath, R.V. Klucas, M. Becana, *Plant Physiol.* 133 (2003) 773–782.
- [14] A. Miller, Superoxide dismutases: ancient enzymes and new insights, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 586 (2012) 585–595.
- [15] L.A. del Río, L.M. Sandalio, D.A. Altomare, B.A. Zilinskas, Mitochondrial and peroxisomal manganese superoxide dismutase: differential expression during leaf senescence, *J. Exp. Bot.* 54 (384) (2003) 923–933.
- [16] C.T. Lin, M.T. Lin, Y.T. Chen, J.F. Shaw, Subunit interaction enhances enzyme activity and stability of sweet potato cytosolic Cu/Zn superoxide dismutase purified by a His-tagged recombinant protein method, *Plant Mol. Biol.* 28 (1995) 303–311, <https://doi.org/10.1007/BF00020249>.
- [17] S. Kanematsu, K. Asada, Chloroplast and cytosol isozymes of Cu, Zn-superoxide dismutase: their characteristic amino acid sequences, *Free Radic. Res. Commun.* 12–13 (1991) 383–390.
- [18] K. Ogawa, S. Kanematsu, K. Asada, Generation of superoxide anion and localization of CuZn-superoxide dismutase in the vascular tissue of spinach hypocotyls: their association with lignification, *Plant Cell Physiol.* 37 (6) (1996) 790–799.
- [19] A. Zafra, M.J. Jiménez-Quesada, J.A. Traverso, F.J. Corpas, J.D. Alché, Peroxisomal localization of CuZn superoxide dismutase in the male reproductive tissues of the olive tree, *Microsc. Microanal.* 18 (S5) (2012) 33–34.
- [20] A.C. Asensio, M. Gil-Monreal, L. Pires, Y. Gogorcena, A. Pedro María, J.F. Moran, Two Fe-superoxide dismutase families respond differently to stress and senescence in legumes, *Plant Physiol.* 169 (2012) 1253–1260.
- [21] Y. Yi, F. Xing, X. Huang, H. Chen, F. Wang, Medicinal plants of *Bulbophyllum* species in China, *J. Trop. Subtropical Bot.* 13 (2005) 65–69.
- [22] P. Boonkorkaew, List of Biodiversity Resource: Thai Orchid Database Biodiversity-Based Economic Development Office, Public organization, Bangkok, 2010.
- [23] R.M.P. Gutiérrez, Orchids: a review of uses in traditional medicine, its phytochemistry and pharmacology, *J. Med. Plants Res.* 4 (8) (2010) 592–638.
- [24] M.M. Hossain, Therapeutic orchids: traditional uses and recent advances—an overview, *Fitoterapia* 82 (2) (2011) 102–140.
- [25] J.M. Kong, N.K. Goh, L.S. Chia, T.F. Chia, Recent advances in traditional plant drugs and orchids, *Acta Pharmacol. Sin.* 24 (1) (2003) 7–21.
- [26] B. Wu, S. He, Y.J. Pan, New dihydrodibenzopin from *Bulbophyllum kwangtungense*, *Planta Med.* 72 (13) (2006) 1244–1247.
- [27] Y.G. Chen, J. Xu, Y. Hong, C. Qing, Y. Zhang, L. Wang, Y. Liu, J. Wang, Cytotoxic phenolics from *Bulbophyllum odoratissimum*, *Food Chem.* 107 (1) (2008) 169–173.
- [28] M.B. Chand, M.R. Paudel, B. Pant, The antioxidant activity of selected wild orchids of Nepal, *J. Coast. Life Med.* 4 (9) (2016) 731–736.
- [29] A. Kalaarasam, Antioxidant properties of medicinal orchid in Indian Vegetation Flora, *Biomed. Nurs.* 2 (4) (2016) 1–14.
- [30] P. Bhattachacharyya, S. Kumaria, P. Tandon, High frequency regeneration protocol for *Dendrobium nobile*: a model tissue culture approach for propagation of medicinally important orchid species, *South Afr. J. Bot.* 104 (2016) 232–243.
- [31] T.L. Palama, I. Fock, Y.H. Choi, R. Verpoorte, H. Kodja, Biological variation of *Vanilla planifolia* leaf metabolome, *Phytochemistry* 71 (5–6) (2010) 567–573.
- [32] B.C. Tan, C.F. Chin, S. Liddell, P. Alderson, Proteomic analysis of callus development in *Vanilla planifolia* Andrews, *Plant Mol. Biol. Rep.* 31 (2013) 1220–1229.
- [33] G. Chen, D. Chen, T. Wang, C. Xu, L. Li, Analysis of the proteins related to browning in leaf culture of *Phalaenopsis*, *Sci. Hortic.* 141 (2012) 17–22.
- [34] K.E.M. Sedeek, W. Qi, M.A. Schauer, A.K. Gupta, L. Poveda, S. Xu, Z.J. Liu, U. Grossniklaus, F.P. Schiest, P.M. Schluter, Transcriptome and proteome data reveal candidate genes for pollinator attraction in sexually deceptive orchids, *PLoS One* 8 (5) (2013) e64621.
- [35] X. Li, W. Xu, M.R. Chowdhury, F. Jin, Comparative proteomic analysis of labellum and inner lateral petals in *Cymbidium ensifolium* flowers, *Int. J. Mol. Sci.* 15 (11) (2014) 19877–19897.
- [36] W. Wang, H. Yu, T. Li, L. Li, G. Zhang, Z. Liu, T. Huang, Y. Zhang, Comparative proteomics analyses of pollination response in endangered orchid species *Dendrobium chrysanthum*, *Int. J. Mol. Sci.* 18 (12) (2017) 2496.
- [37] R.B.S. Valadares, S. Perotto, E. Santos, M. Lambais, Proteome changes in *Oncidium sphacelatum* (Orchidaceae) at different trophic stages of symbiotic germination, *Mycorrhiza* 24 (5) (2014) 349–360.
- [38] M.Y. López-Chávez, K. Guillén-Navarro, V. Bertolini, S. Encarnación, M. Hernández-Ortiz, K. Guillén-Navarro, V. Bertolini, S. Magdalena, I. Sánchez-Moreno, A. Damon, Proteomic and morphometric study of the in vitro interaction between *Oncidium sphacelatum* Lindl. (Orchidaceae) and *thanatephorus* sp. (ceratobasidiaceae), *Mycorrhiza* 26 (5) (2016) 353–365.
- [39] X.B. Xu, X.Y. Ma, H.H. Lei, H.M. Song, Q.C. Ying, M.J. Xu, S.B. Liu, H.Z. Wang, Proteomic analysis reveals the mechanisms of *Mycena dendrobii* promoting transplantation survival and growth of tissue culture seedlings of *Dendrobium officinale*, *J. Appl. Microbiol.* 18 (2015) 1444–1455.
- [40] J. Chen, S.S. Liu, A. Kohler, B. Yan, H.M. Luo, X.M. Chen, S.X. Guo, iTRAQ and RNA-seq analyses provide new insights into regulation mechanism of symbiotic germination of *Dendrobium officinale* seeds (Orchidaceae), *J. Proteome Res.* 16 (6) (2017) 2174–2187.
- [41] S. Feng, K. Jiao, H. Guo, M. Jiang, J. Hao, H. Wang, C. Shen, Succinyl-proteome profiling of *Dendrobium officinale*, an important traditional Chinese orchid herb, revealed involvement of succinylation in the glycolysis pathway, *BMC Genom.* 18 (2017) 598.
- [42] J.W. Li, X.D. Chen, X.Y. Hu, L. Ma, S.B. Zhang, Comparative physiological and proteomic analyses reveal different adaptive strategies by *Cymbidium sinense* and *C. tracyanum* to drought, *Planta* 247 (2018) 69–97.
- [43] H. Herrera, R. Valadares, G. Oliveira, A. Fuentes, L. Almonacid, S.V. do Nascimento, Y. Bashan, C. Arriagada, Adaptation and tolerance mechanisms developed by mycorrhizal *Bipinnula fimbriata* plantlets (Orchidaceae) in a heavy metal-polluted ecosystem, *Mycorrhiza* 28 (7) (2018) 651–663.
- [44] F.T. van Amsterdam, A. Roveri, M. Maiorino, E. Ratti, F. Ursini, Lacidipine: a dihydroxyridine calcium antagonist with antioxidant activity, *Free Radic. Biol. Med.* 12 (3) (1992) 183–187.
- [45] W.J. Hurlkman, C.K. Tanaka, Solubilization of plant membrane proteins for analysis by two dimension gel electrophoresis, *Plant Physiol.* 81 (1986) 802–806.
- [46] S.B. Carpentier, E. Witters, K. Laukens, P. Deckers, R. Swennen, B. Panis, Preparation of protein extracts from recalcitrant plant tissues: an evaluation of different methods for two-dimensional gel electrophoresis analysis, *Proteomics* 5 (10) (2005) 2497–2507.
- [47] T. Koenig, B.H. Menze, M. Kirchner, F. Monigatti, K.C. Parker, T. Patterson, J.J. Steen, F.A. Hamprecht, H. Steen, Robust prediction of the MASCOT score for an improved quality assessment in mass spectrometric proteomics, *J. Proteome Res.* 7 (2008) 3708–3717.
- [48] T.Z. Kristiansen, J. Bunkenborg, M. Gronborg, H. Molina, P.J. Thuluvath, P. Argani, M.G. Goggins, A. Maitra, A. Pandey, A proteomic analysis of human bile, *Mol. Cell. Proteomics* 3 (7) (2004) 715–728.
- [49] R.P. Huntley, T. Sawford, P. Mutwogo-Mueller, A. Shypitsyna, C. Bonilla, M.J. Martin, C. O'Donovan, The GO database: gene Ontology annotation updates for 2015, *Nucleic Acids Res.* 34 (D1) (2014) D1057–D1063.
- [50] K. Hinkelmann, O. Kempthorne, Design and Analysis of Experiments: Volume I, Introduction to Experimental Design, second ed., John Wiley and sons, New Jersey, 2008.
- [51] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Anal. Biochem.* 72 (1–2) (1976) 248–254.
- [52] R. Khanna-Chopra, S. Sabarinath, Heat-stable chloroplastic Cu/Zn superoxide dismutase in *Chenopodium murale*, *Biochem. Biophys. Res. Commun.* 320 (4) (2004) 1187–1192.
- [53] A. Chandrasekaran, M.D.P.S. Idelchick, J.A. Melendez, Redox control of senescence and age-related disease, *Redox Biology* 11 (2017) 91–102.
- [54] M. Krishnan, H.T. Nguyen, J.J. Burke, Heat shock protein synthesis and thermal tolerance in wheat, *Plant Physiol.* 90 (1989) 140–145.
- [55] W. Wang, B. Vinocur, O. Shoseyov, A. Altman, Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response, *Trends Plant Sci.* 9 (5) (2004) 244–252.
- [56] J. Chen, T. Gao, S. Wan, Y. Zhang, J. Yang, Y. Yu, W. Wang, Genome-wide identification, classification and expression analysis of the HSP gene superfamily in tea plant (*Camellia sinensis*), *Int. J. Mol. Sci.* 19 (9) (2018) 2633.
- [57] G.F. Wang, X. Wei, R. Fan, H. Zhou, X. Wang, C. Yu, L. Dong, Z. Dong, X. Wang, Z. Kang, H. Ling, Q.H. Shen, D. Wang, X. Zhang, Molecular analysis of common wheat genes encoding three types of cytosolic heat shock protein 90 (Hsp 90): functional involvement of cytosolic Hsp90s in the control of wheat seedling growth and disease resistance, *New Phytol.* 191 (2011) 418–431.
- [58] L.D. Noel, G. Cagna, J. Stuttmann, L. Wirthmueller, S. Betuyaku, C.P. Witte, R. Bhat, N. Pochon, T. Colby, J.E. Parker, Interaction between SGT1 and cytosolic/nuclear HSC70 chaperones, *Plant Cell* 19 (2007) 4061–4076.
- [59] A. Zafra, A.J. Castro, J. Alché, Identification of novel superoxide dismutase isoenzymes in the olive (*Olea europaea* L.) pollen, *BMC Plant Biol.* 18 (2018) 114–120.
- [60] C. Galeja, L. Barros, A.L. Antonio, M.B.P. Oliveira, I.C. Ferreira, A comparative study between natural and synthetic antioxidants: evaluation of their performance after incorporation into biscuits, *Food Chem.* 216 (2017) 342–346.
- [61] A.A. Hübner, A.V. Neto, F. Sobreira, C. Pinto, M.F. Dario, F.R. Lourenço, A.R. Baby, E.M. Bacchi, Phytochemistry, antioxidant activity, and sunscreen efficacy of hydroethanolic extract of Cabernet Sauvignon grape pomace (*Vitis vinifera* L.), *Planta Med.* 82 (2016) S1–S381 S 01.
- [62] V. Lobo, A. Patil, A. Phatak, N. Chandra, Free radicals, antioxidants and functional foods: impact on human health, *Phcog. Rev.* 4 (8) (2010) 118–126.
- [63] S. Furuta, Y. Nishiba, I. Suda, Fluorometric assay for screening anti-oxidative activity of vegetables, *J. Food Sci.* 62 (3) (1997) 526–528.
- [64] M. Chinsamy, J.F. Finnie, J. Van Staden, Anti-inflammatory, antioxidant, anticholinesterase activity and mutagenicity of South African medicinal orchids, *South Africa of Botany* 91 (2014) 88–98.