Copper excess promotes propagation and induces proteomic change in root cultures of Hyoscyamus albus L.

Author(s)
Sako, Ari; Kandakar, Jebunnahar; Tamari, Noriko; Higa, Ataru; Yamaguchi, Kenichi; Kitamura, Yoshie

Citation
Plant Physiology and Biochemistry, 103, pp.1-9; 2016

Issue Date
2016-06

URL
http://hdl.handle.net/10069/36243

Copyright
© 2016 Elsevier Masson SAS.; This manuscript version is made available under the CC-BY-NC-ND 4.0 license.
Copper excess promotes propagation and induces proteomic change in root cultures of *Hyoscyamus albus* L.

Ari Sako\(^a\) · Jebunnahar Kandakar\(^b\) · Noriko Tamari\(^b\) · Ataru Higa\(^b\) · Kenichi Yamaguchi\(^b\) \(^c\)
· Yoshie Kitamura\(^a,\(^b\)*

\(^a\) Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Nagasaki 852-8521, Japan

\(^b\) Graduate School of Science and Technology, Nagasaki University, Nagasaki 852-8521, Japan

\(^c\) Division of Biochemistry, Faculty of Fisheries, Nagasaki University, Nagasaki 852-8521, Japan

*Corresponding author:
Yoshie Kitamura
Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Nagasaki 852-8521, Japan.
Tel/Fax: +81-95-819-2759
E-mail: k-yoshie@nagasaki-u.ac.jp
Abstract

*Hyoscyamus albus* L. seedlings respond positively to copper (Cu) excess. In the present study, to understand how roots cope with Cu excess, propagation and proteome composition in the presence of Cu were examined using a root culture system. When *H. albus* roots were cultured in a medium without Cu, root growth deteriorated. However, in the presence of Cu, root growth increased in a concentration-dependent manner, and vigorous lateral root development was observed at 200 µM Cu. Cu accumulation in the roots increased with the Cu supply. Subcellular fractionation revealed that the highest amount of Cu was present in the cell wall-containing fraction, followed by the soluble fraction. However, the highest specific incorporation of Cu, in terms of fresh weight, was in the mitochondria-rich fraction. High Cu levels enhanced respiration activity. Comparative proteomic analysis revealed that proteins involved in carbohydrate metabolism, *de novo* protein synthesis, cell division, and ATP synthesis increased in abundance, whereas the proteasome decreased. These results indicate that Cu promotes propagation of *H. albus* roots through the activation of the energy supply and anabolism. Newly propagated root tissues and newly generated proteins that bind to Cu may provide space and reservoirs for deposition of additional Cu.

**Keywords:** copper; heavy metal; *Hyoscyamus albus*; proteomics; root culture; root respiration.
1. Introduction

*Hyoscyamus* spp. (Solanaceae) are well-known medicinal plants that produce tropane alkaloids, such as atropine (dl-hyoscyamine) and scopolamine, which are commercially important anticholinergic and sedative drugs (Evans, 2009). In a previous study, we determined that *Hyoscyamus albus* L. roots can survive iron (Fe) deficiency and that, under Fe-deficient conditions, they secrete riboflavin into the rhizosphere (Higa et al., 2010; 2012). To determine whether this phenomenon was specific to Fe deficiency, we examined the effect on root growth of deficiency in other essential metals, including manganese, zinc (Zn), molybdenum, and copper (Cu). We observed that the elimination of Cu seriously impaired growth, suggesting that *H. albus* roots are sensitive to Cu deficiency. A further study revealed that *H. albus* seedlings are tolerant to excess Cu (Tamari et al., 2014).

Cu is an essential heavy metal for both plants and humans. In plants, Cu is involved in a wide range of redox reactions that are essential for photosynthesis, respiration, ethylene perception, reactive oxygen metabolism, and cell-wall remodelling via Cu-containing proteins such as plastocyanin, Cu/Zn superoxide dismutase, and cytochrome c oxidase (Burkhead et al., 2009). The Cu content of nonpolluted soils worldwide ranges from 1 to 140 mg.kg$^{-1}$ dry weight, and Cu usually occurs in the form of poorly mobile compounds incorporated into carbonates, sulphates, organic matter, and clays (Terelak and Motowicka-Terelak, 2000). However, Cu in its free form is very reactive in the presence of both thiols and oxygen and can promote oxidative stress (Ravet and Pilon, 2013; Sáez et al., 2015); therefore Cu excess is toxic to both animals and plants. Furthermore, the widespread industrial and agricultural uses of Cu cause serious Cu pollution, resulting in an increase in Cu mobility and bioavailability (Martins et al., 2012; Zou et al., 2015). A concentration of 3 μM or higher is considered a high level of Cu (Bernal et al., 2006). Some plants, denoted as Cu-excluders, survive by limiting Cu uptake and accumulation, whereas others that actively uptake Cu, rapidly transport it, and then efficiently accumulate Cu in shoots are defined as accumulators/hyperaccumulators (Masarovičová et al., 2010; Visioli and Marmiroli, 2013). In our previous study, we reported that *H. albus* seedlings are accumulators of Cu (Tamari et al., 2014).

As plants usually take up Cu through their roots, the roots themselves must be tolerant or resistant to high Cu concentrations. This tolerance or resistance is maintained by biological processes in which functional proteins play an important role. In order to better understand the molecular mechanisms underlying Cu tolerance, proteomic studies have been performed using various roots exposed to excess Cu, including *Agrostis capillaris* L. (Hego et al., 2014).
Arabidopsis thaliana (L.) Heynh. (Kung et al., 2006), Cannabis sativa L. (Bona et al., 2007), Elsholtzia splendens Nakai (Liu et al., 2014), rice (Oryza sativa L.) (Chen et al., 2015; Song et al., 2013), and wheat (Triticum aestivum L.) (Li et al., 2013).

The aim of the present study was to understand how roots cope with Cu excess. We examined how Cu directly affects the propagation of cultured roots of H. albus, the locations to which Cu is allocated in the roots, and the biochemical and proteomic changes that are induced by Cu excess. In addition, we compared the proteome composition obtained in our previous study of H. albus roots subjected to Fe deficiency stress (Khandakar et al., 2013) and data reported in previous proteomic studies of other plant roots under Cu excess (Bona et al., 2007; Chen et al., 2015; Hego et al., 2014; Kung et al., 2006; Li et al., 2013) with the data from the present study to determine the novelty and/or generality of stress responses of H. albus roots.

2. Materials and Methods

2.1. Root materials and culture conditions
Root cultures of H. albus used in this study were established following Higa et al. (2008). Roots were maintained on MS basal medium (Murashige and Skoog, 1962) solidified with 0.2% (w/v) gellan gum. A primary root tip with a few lateral roots (approximately 2 cm in length), isolated from a 2-week-old root culture, was either directly cultured in test medium or pre-cultured in MS medium containing 1% (w/v) sucrose for 2 weeks and then transferred to test medium and cultured for 1 additional week. MS media containing different Cu concentrations (0, 0.1, 1, 20, and 200 µM), prepared by either elimination or addition of CuSO₄, were used as test media. EDTANa₂ (Dojindo Co., Japan), a chelating agent, was added to the 20 and 200 µM Cu media to prevent Cu precipitation. Media were autoclaved at 121 ºC for 15 min before use. All cultures were maintained in 100 mL and 50 mL conical flasks containing 25 mL and 15 mL of liquid medium, respectively, and incubated under agitation (80 rpm) at 25 ºC, in the dark. The cultures were harvested by vacuum filtration and washed with distilled water. Freshly collected roots were weighed and dried at 50 ºC overnight prior to analyses of root mass and amount of Cu in the roots (dry weight basis). Only fresh roots were used for fractionation analysis.

Following the protocol in a previous report for comparative proteomic analysis, root cultures were pre-propagated in B5 medium (Gamborg et al., 1968) containing 1% (w/v) sucrose for 2 weeks. Roots were then separated into sub-sets and transferred to fresh B5 medium containing 1% (w/v) sucrose, either with normal Cu concentration (0.1 µM) or with
high Cu concentration (200 µM). Root cultures were maintained for 5 days. Root tips were harvested at day 5, frozen in liquid nitrogen, and stored at -80 ºC until further use.

2.2. Proteomic analysis

Proteins were extracted from root tips (100 mg fresh weight) using bead beating followed by the acid guanidinium–phenol–chloroform method (Khandakar et al., 2013). The same methodological approach used in a previous small-scale proteomic analysis (Khandakar et al., 2013) was applied in the present study. In brief, 2-D gel electrophoresis (20 µg protein/2-D gel) was performed with a 7-cm ReadyStrip™ IPG Strip (73-mm-long, 3.3-mm-wide, and 0.5-mm-thick rehydrated strip with a linear pH gradient of 5–8; Bio-Rad, Hercules, CA, USA) for the 1st dimension and a Mini-PROTEAN® TGX™ gel AnykD (IPG/prep, 72-mm-long, 86-mm-wide, and 1-mm-thick gel; Bio-Rad) for the 2nd dimension. Gels were visualised with Flamingo® fluorescent staining (Bio-Rad). Image analysis, spot detection, statistical analysis, and protein in-gel digestion, followed by protein identification using matrix-assisted laser desorption/ionization quadrupole ion trap time-of-flight (MALDI-QIT-TOF) mass spectrometry and determination of pl, MW, and subcellular localisation were performed by following the methods described in our previous paper (Khandakar et al., 2013). Cross-species protein identification was performed by MS/MS ion search using MASCOT® version 2.3 (Matrix Science, London, UK). MS/MS spectra were processed with MASCOT Distiller™ version 2.3 (Matrix Science, London, UK), and resulting peak lists were searched against the SwissProt 2015_12 database (550,116 sequences and 196,219,159 residues; taxonomy: Viridiplantae, 37,197 sequences) and the EST_Solanaceae 2015_12 database (7,984,062 sequences and 1,484,673,610 residues) in our own MASCOT server. Search parameters were: trypsin (enzyme), carbamidomethyl (Cys; fixed modifications), oxidation (M; variable modifications), monoisotopic (mass values), ± 0.5 Da (peptide mass tolerance), ± 0.3 Da (fragment mass tolerance) and 1 (maximum missed cleavage). The identification was considered positive when the assigned MASCOT score was above threshold level ($p < 0.05$) (at least two peptides, protein score > 60; single peptide, peptide score > 48; annotated MS/MS spectra of the MASCOT peptide view are shown in Supplemental Data 1), sequence coverage was > 1.5%, and theoretical and observed mass and pl values were similar.

2.3. Fractionation

*H. albus* fresh root tissues (approximately 1 g) were fractionated according to the method reported previously (Tamari et al., 2014). Fresh weight (FW) of the cell-wall containing fraction
was measured after collection by nylon mesh filtration (82 µm, *in vacuo*) and washing twice with extraction buffer containing 50 mM Tris-HCl (pH 7.5), 250 mM sucrose, and 10 mM dithiothreitol. The sedimentation fractions, which included the nucleus plus plastid-rich and mitochondria-rich fractions, were isolated by centrifugation at 880 × *g* for 15 min, followed by 21,880 × *g* for 30 min. Their FWs were obtained by weighing empty centrifuge tubes, adding filtrate or supernatant to the tubes, and removing the supernatants after centrifugation. The tubes with sediments were again weighed, and the weights of the empty centrifuge tubes were subtracted from these values. FW of the remaining supernatant (considered as soluble fraction) was calculated by subtracting the weight of the sum of three fractions (the cell wall-containing fraction, the nucleus plus plastid-rich fraction, and the mitochondria-rich fraction) from that of the starting materials. The ratio (%) of FW in each fraction was calculated by dividing by the sum of FW in four fractions: the cell wall-containing fraction, the nucleus plus plastid rich-fraction, the mitochondria-rich fraction, and the soluble fraction. The ratio (%) of Cu amounts in each fraction was obtained in the same manner as that of FW.

2.4. Measurement of amount of Cu

Dried tissues and fractions were digested with 60% (w/v) HNO₃ in a microwave oven (Perkin Elmer Multiwave, MA, USA) at 160 ºC for 20 min. The amount of Cu was determined by atomic absorption spectroscopy (Hitachi Z-2000, Tokyo, Japan) based on a calibration curve prepared with a Cu standard solution (Wako Chemical, Osaka, Japan).

2.5. Assay of TTC-reducing activity

A 2,3,5-triphenyltetrazolium chloride (TTC)-reducing assay was used to determine respiration activity (Higa et al., 2010). Briefly, the medium was removed from the root culture using a pipette, roots were washed with 5 mL sterile water, and 10 mL filter-sterilised TTC reagent (Sigma) was added. The prepared TTC reagent was 0.5% (w/v) TTC in 50 mM potassium phosphate buffer (pH 7.0). Incubation with TTC reagent was performed under sterile conditions for 3 h with shaking (80 rpm) to allow the reagent to permeate uniformly.

After incubation, roots (50–150 mg) harvested by vacuum filtration were ground in liquid nitrogen to a powder with a mortar and a pestle. The powder was transferred to a centrifuge tube and extracted with 3 mL of 95% (v/v) ethanol for 15 min in a water bath at 60 ºC. After centrifugation (880 × *g*; 15 min), the absorbance of the supernatant was recorded at 520 nm. As the absorbance of denatured roots boiled for 10 min was 0.020 ± 0.001 at 520 nm, 95% (v/v) ethanol was used as control and 0.02 was subtracted from the absorbance value. Reduction activity was calculated using a standard curve previously obtained using authentic
1,3,5-triphenylformazan (Tokyo Chemical Industry Co., Tokyo, Japan) dissolved in 95% (v/v) ethanol.

2.6. Statistical analysis

All the experiments were performed using at least three replicates, and the means and standard deviations were calculated. The statistical differences were analysed by Student t-test or analysis of variance (ANOVA) using Excel Statistics software (Social Survey Research Information Co., Tokyo, Japan). Significant differences were expressed as \( p < 0.05 \) and \( p < 0.01 \).

3. Results

3.1. Effect of Cu concentration on root propagation

In order to establish the response of \( H. \ albus \) roots to Cu, root tips were inoculated into preparations of liquid MS media containing Cu at various concentrations (0.1, 1, 20, and 200 µM), as well as into Cu-free MS medium. Roots were then cultured for 7 and 14 days in the dark. Roots grew well at all Cu concentrations but not under the Cu-deficient condition. Root propagation was promoted at the highest Cu concentration. In particular, lateral root development was noticeably different at 200 µM Cu than at 0.1 µM, the normal Cu concentration (Fig. 1). Although there was no significant difference in the number of lateral roots, the length of these roots and particularly the distance from the main root tip to the first initiated lateral root differed significantly between treatments (Table 1). At 200 µM Cu, the distance from the main root tip was two-thirds that at 0.1 µM Cu. Root growth measured as dry weight (DW) was significantly suppressed under Cu-deficient conditions compared with that at 0.1 µM Cu (reduced from 5.9 ± 1.7 mg per culture to 2.3 ± 1.5 mg per culture after 14 days). However, after 14 days, root growth was enhanced when Cu was added, reaching the highest value at 200 µM Cu (11.6 ± 2.3 mg per culture) (Fig. 2). The effects of 200 µM Cu and Cu deficiency on root growth were also clearly seen after 7 days of culture, although the effects were not obvious at the other concentrations.

3.2. Cu accumulation in roots

As \( H. \ albus \) roots grew well under Cu-rich conditions (see section 3.1), root tissues cultured for 2 weeks in media containing 0.1, 20, or 200 µM Cu were analysed by atomic absorption
spectrometry. Cu accumulation increased with the increasing Cu concentration (Table 2). At a concentration of 200 µM Cu, the accumulation of Cu was about 14 times higher than that at the lowest concentration.

The bioaccumulation/bioconcentration factor (BF) has been proposed as a standard to estimate the ability of plants to take up arsenic from the rhizosphere (Masarovičová et al., 2010). In hydroponics, the ratio of metal concentration in plant dry mass (µg.g\(^{-1}\) DW) to the external solution (µg.cm\(^{-3}\)) is used as a measure of BF. Our results showed that BF values of *H. albus* roots varied from 2,700 at the lowest Cu concentration to 19 at the highest.

The high levels of Cu incorporation into *H. albus* roots suggested that Cu might be accumulated at the subcellular level. To test whether this was occurring, root tissues were divided into four fractions by filtration and centrifugation: a cell wall-containing fraction, a fraction containing the nuclei plus plastids, a mitochondria-rich fraction, and a soluble fraction, including vacuoles and cytosol. Cu incorporation at 0.1 µM Cu occurred mostly in the cell-wall containing (approximately 39%) and soluble (approximately 37%) fractions, followed by the nucleus and plastid-rich fraction (approximately 13%) and the mitochondria-rich fraction (approximately 11%). At 200 µM Cu, Cu content increased in all fractions, but particularly in the cell wall-containing fraction (approximately 62%), followed by the soluble fraction (approximately 31%) (Table 3). The total amount of Cu recovered from the cell wall-containing fraction was more than 20 times as high for the 200 µM Cu treatment as for the 0.1 µM Cu concentration.

Interestingly, the calculation of the amount of Cu incorporated into each fraction relative to the fresh weight fraction (i.e. specific incorporation) showed that the highest specific incorporation was into the mitochondria-rich fraction, both at 0.1 µM and at 200 µM Cu. Moreover, the specific incorporation into this fraction increased over 16-fold between the 200 and the 0.1 µM Cu concentrations (Table 3). Relatively high specific incorporation of Cu into the nucleus and plastid-rich fraction was also observed.

### 3.3. Effect of Cu on root respiration

As root growth was enhanced by a concentration of 200 µM Cu (Fig. 2) and the mitochondria-rich fraction showed a high specific incorporation of Cu (Table 3), we anticipated that respiration activity would increase under conditions of Cu excess. To confirm whether this occurred, we examined the effect of Cu concentration (0.1 and 200 µM) on respiration activity, using the TTC-reducing assay. Results showed that respiration activity increased when the Cu concentration increased from 0.1 to 200 µM (\(p < 0.05\)) (Fig. 3), matching our predictions.
3.4. Protein profile under different Cu concentrations

In order to determine which proteins in *H. albus* roots were responsible for enhanced root propagation and higher Cu accumulation under Cu excess, comparative proteomic analysis was performed. Proteins were extracted from 100 mg fresh roots cultured in 0.1 and 200 µM Cu concentrations using CHAPS-urea solution. The total amount of soluble proteins in each treatment was not statistically different (Table 4). Two-dimensional gel electrophoresis of 20 µg of protein revealed more than 200 spots in each sample (Table 4, Fig. 4). Differential protein accumulation between the two Cu concentration treatments was analysed after normalisation using Prodigy SameSpots®, and a threshold value (ANOVA at *p* < 0.05) was set at ≥1.5-fold change. Thirty-four spots were detected, of which 21 increased and 13 decreased in abundance under Cu excess (Table 4).

These 34 spots were digested and peptides were extracted. Peptide solutions were then analysed by MALDI-QIT-TOF mass spectrometry. In total, 22 proteins were identified, of which 16 increased in abundance under Cu excess and the remainder decreased (Table 5; Supplemental Data 2). According to the previous classification based on their function (Khandakar et al., 2013), proteins were divided into five groups: carbohydrate metabolism (6 proteins, 27%), amino acid/protein metabolism (6 proteins, 27%), defence response (4 proteins, 18%), ATP-related metabolism (4 proteins, 18%), and other functions (2 proteins, 10%). Only 1 protein per group decreased in abundance under Cu excess and all the others increased, except in the defence response group, where 2 proteins decreased in abundance (Table 5).

Under Cu excess, 5 proteins involved in carbohydrate metabolism increased in abundance: 2 isozymes of pyrophosphate-fructose 6-phosphate and 1-phosphotransferase β-subunit (spots 13 and 74), enolase (spot 63) from the glycolytic pathway, NADP-dependent isocitrate dehydrogenase (ISD, spot 2), and a dihydrolipoamide dehydrogenase precursor (spot 17) from the TCA cycle. Also in this group, fructose-bisphosphate aldolase-like protein (spot 67) decreased in abundance. In the amino acid/protein metabolism group, 5 proteins increased in accumulation: ferredoxin-nitrite reductase (NiR, spot 4), which is responsible for assimilation of NO₂⁻; the mitochondrial elongation factor Tu (EF-Tu) (spot 8), which is part of the mechanism that synthesises new proteins by translation at the ribosome; and 3 isozymes of S-adenosylmethionine (SAM) synthase (spots 6, 7 and 18), which catalyse the biosynthesis of SAM from methionine and ATP. The proteasome subunit β type-6 protein (spot 47), characterised by its ability to cleave peptides, decreased in abundance. Proteins related to ATP metabolism, such as the vacuolar H⁺-ATPase A1 subunit isoform (spot 57), the ATP synthase...
subunit α (spot 39), and the adenosine kinase (ADK) isoform (spot 9) increased in abundance, whereas the UMP/CMP kinase-like 1S (spot 46) decreased. Defence-response proteins that increased in abundance were the peroxidase 27 precursor putative (spot 1) and the heat shock cognate 70-kDa protein (HSP 70, spot 10), while the superoxide dismutase (SOD) [Fe] (spot 72) and the glutathione peroxidase (GPX, spot 61) decreased. Proteins belonging to the general group (other functions), such as the cell division control protein 48 homolog A (CDC, spot 12), which is involved in cell division and growth processes (Feiler et al., 1995), increased in abundance, but the soluble inorganic pyrophosphatase (PPA) decreased (spot 49). More than 2.0-fold increases in accumulation were observed in ISD (2.7-fold), peroxidase 27 (2.5-fold), NiR (2.2-fold), and vacuolar H⁺-ATPase (2.1-fold).

4. Discussion

Supplying different concentrations of Cu (from 0 to 200 µM) to the roots of *H. albus* revealed that Cu deficiency causes serious growth impairment and Cu excess promotes root development (Table 1, Figs. 1 and 2), especially lateral root elongation, although it inhibits axis root elongation (Table 1, Fig. 1). Similar inhibition of axis root elongation, as well as enhancement of lateral root formation, in response to Cu excess was observed in *A. thaliana* (Lequeux et al., 2010; Potters et al., 2007; Wojcik and Tukiendorf, 2003). Meanwhile, lateral root length and total root weight sharply decreased under Cu excess, even at concentrations below 50 µM. Lateral elongation of *H. albus* roots was enhanced, and root weight also increased (Figs. 1 and 2, Table 1), indicating that roots thrive under Cu excess.

Comparative proteomic analysis revealed that, under conditions of Cu excess, cell division and *de novo* syntheses of amino acids and proteins were enhanced, whereas protein degradation was depressed, suggesting that the roots of *H. albus* actively grow when Cu is abundant. To support this active root growth, the energy supply in the roots was also enhanced, as shown by the increased accumulation of proteins involved in glycolysis, the TCA cycle, and the electron transport chain (ETC) (Table 5). These results coincided with the observed increase in respiration activity under Cu excess (Fig. 4). Cu excess may be involved in lateral root development either by activating hormones such as abscisic acid (ABA) (Gibson et al., 2012) or suppressing the production of new hormones such as strigolactones, which inhibit lateral root elongation. Although these results have been reported in *A. thaliana* (Kapulnik et al., 2011), the proteomic profile from the present study did not show support for these mechanisms in *H.*
Among the 22 proteins identified in this study, only 4 proteins (spots 1, 39, 49, and 61) were found in a previous proteomic analysis in H. albus roots subjected to Fe deficiency (Khandakar et al., 2013). Interestingly, soluble PPA (spot 49) and GPX (spot 61) decreased, and ATP synthase subunit α (spot 39) increased under Cu excess, whereas opposite results were obtained under Fe deficiency, except for the peroxidase 27 precursor putative (spot 1), which increased in both cases. Although H. albus roots are tolerant to Fe deficiency, they appear to respond with energetic and Fe-conservation strategies to severe Fe restriction. There may be a shift between de novo synthesis and enhanced reutilisation of amino acids produced by proteolysis (Khandakar et al., 2013). Under Cu excess, increased accumulations of ATP-involved proteins, including three SAM synthase isozymes (spots 6, 18, and 7), vacuolar H⁺-ATPase (spot 57), ATP synthase subunit α (spot 39), ADK (spot 9), and CDC (spot 12), indicated active consumption and production of ATP. In addition, active de novo synthesis of amino acids and proteins seems to occur, as shown by the increased accumulation of NiR (spot 4) and mitochondrial EF-Tu (spot 8). Elevated levels of EF-Ts, the guanine nucleotide-exchange factor for EF-Tu, also occurred specifically in response to Cu in Pseudomonas putida Trevisan KT2440, suggesting that protein synthesis increases in response to Cu (Miller et al., 2009). In Vibrio parahaemolyticus, EF-Tu and EF-Ts were detected by secretomics and these proteins were abundant in strains that were tolerant to heavy metals (Cd and Cu) (He et al., 2015). On the other hand, proteolytic activity seems to decrease in the presence of heavy metals, as shown by the decrease in proteasome subunit (spot 47). A significant decrease in a proteasome was also observed in the marine brown algae Sargassum fusiforme (Harv.) Setchell when it was exposed to chronic Cu stress (Zou et al., 2015).

The proteomic profile of H. albus roots subjected to Cu excess was not consistent with previous results obtained using the roots of various plants (Bona et al., 2007; Hego et al., 2014; Li et al., 2013; Song et al., 2013). When the roots of wheat seedlings were exposed to 100 µM Cu, 3 proteins that are functionally similar to those found in H. albus roots were identified: HSP 70, SAM synthase 1, and ATP synthase α (Li et al., 2013). In wheat roots, HSP 70 and SAM synthase decreased, whereas in H. albus roots, both proteins increased. Nevertheless, the accumulation of ATP synthase increased in the roots of both species. In the roots of a Cu-tolerant rice variety exposed to 8 µM Cu (Song et al., 2013), SAM synthase and HSP 80 increased in abundance, similar to our observations in H. albus. C. sativa roots supplied with 600 µM Cu showed a decrease in enolase (Bona et al., 2007), while H. albus roots showed an increase in this protein. Likewise, enolase and ATP synthase α were reduced in roots of
metallicolous populations of *A. capillaris* in response to ~30 µM Cu (Hego et al., 2014), but opposite results were obtained in *H. albus* roots. These findings might be due to the different mechanisms used by different plant species to deal with excess Cu, the dose and period of Cu supply, or the age of plant materials.

*H. albus* roots actively accumulate Cu: the BF values of the roots varied between 2,700 (at 0.1 µM Cu) and 19 (at 200 µM Cu) (Table 2). BF values of excluders, accumulators, and hyperaccumulators are < 1, > 1, and > 10, respectively (Masarovičová et al., 2010). Therefore, *H. albus* roots take up Cu actively from the rhizosphere, especially at low Cu concentrations. Results suggest that *H. albus* roots need Cu for growth, an idea supported by the observations of severe growth impairment when the species is maintained under Cu-deficient conditions and of better root growth in the presence of excess Cu (Table 1, Figs. 1 and 2).

Cu accumulation in *H. albus* root cells occurred mainly in the cell-wall containing and the soluble fractions (Table 3). As vacuoles were included in the soluble fraction, these results seem consistent with the common response of plants to toxic metals. In bean (*Phaseolus vulgaris* L.) leaves, the cell wall was the major site of Cu ion accumulation (Bouazizi et al., 2011). Similarly, root cells of *Bechmeria nivea* (L.) Gaud. accumulated approximately 50% of total cadmium in the cell wall, followed by 37% in the soluble fraction (Wang et al., 2008). In the present study, the response to Cu excess (Table 3) was similar to the result in the *B. nivea* study; unlike previous studies, we used the roots of *H. albus* seedlings in which the soluble fraction contained higher amounts of Cu than the cell wall-containing fraction (Tamari et al., 2014). As cultured roots of *H. albus* are only able to transport Cu within root cells, Cu may be retained in apoplasts after it reaches saturation inside the cells. Vacuoles (classed as a soluble fraction) must be the main active site of Cu sequestration inside the cells, preventing Cu toxicity in the cytosol (Bernal et al., 2006). Membrane proteins that actively transport Cu, such as Cu-ATPases (Migocka, 2015) and copper transporter (Yu et al., 2014), could not be identified in the present study because only CHAPS-urea soluble proteins were analysed. Most proteins identified in this study exhibited negative values of GRAVY (grand average of hydropathy) (Table 5).

Vacuolar H⁺-ATPase (spot 57) found in the present study seems to be linked to Cu accumulation in organelles such as vacuoles and mitochondria. Eide et al. (Eide et al., 1993) demonstrated that vacuolar H⁺-ATPase was required for efficient Cu detoxification in *Saccharomyces cerevisiae*. This detoxification probably occurred in the vacuoles and the H⁺-ATPase also probably played an important role in mitochondrial respiration. In *Vitis vinifera* L. cells, Cu compartmentation in the vacuole was dependent on the transmembrane pH gradient generated by vacuolar H⁺-ATPase (Martins et al., 2012).
Recent proteomic analysis in *O. sativa* roots (Chen et al., 2015) and *A. thaliana* seedlings (Kung et al., 2006) exposed to excess Cu, using affinity chromatography, revealed that ADK, SAM, ISD, EF-2, and enolase are possible Cu-binding proteins. The enhanced accumulation of ADK, SAM, ISD, and enolase in *H. albus* roots subjected to Cu excess suggests that these proteins seem to function as Cu sinks in the cytoplasm.

A new finding of the present study is the notably high specific incorporation of Cu in the mitochondria that was observed in the 200 µM Cu treatment (Table 3). The mitochondrial respiratory component Complex IV (cytochrome *c* oxidase) contains two Cu centres and cytochromes as co-factors (Rasmusson and Browse, 2002). Therefore, an excess of Cu may result in the deposition of more Cu in Complex IV, which acts as a Cu sink. In fact, plastocyanin in the leaves of *A. thaliana* acts as a Cu sink when large amounts of Cu are available, in addition to its role as an electron carrier (Abdel-Ghany, 2009). In *H. albus* roots, increased accumulation of mitochondrial EF-Tu in response to Cu excess might facilitate the activation of Complex IV, as a consequence of protein synthesis activation in the mitochondria. In fact, respiration activity in *H. albus* root cultures was enhanced under Cu excess (Fig. 3).

Under Cu excess, many proteins involved in the defence response increased in *T. aestivum* (Li et al., 2013) and *O. sativa* (Chen et al., 2015; Song et al., 2013). This response was expected because an excess of Cu results in oxidative stress (Ravet and Pilon, 2013). However, in *H. albus* roots, increases were observed in peroxidase 27 (2.5-fold) and HSP 70 (1.5-fold), but decreases were seen in SOD [Fe] (-1.5-fold) and GPX (-1.5-fold). The function of peroxidase 27 is not yet known, but this protein increases under both Fe deficiency (Khandakar et al., 2013) and Cu excess (Table 5). It may become an important stress biomarker in *H. albus* roots.

5. Conclusions

*H. albus* seedlings tolerate excess Cu (Tamari et al., 2014). In the present study, *H. albus* roots, especially lateral roots, grew vigorously under Cu excess and accumulated large amounts of Cu. At the subcellular level, the total Cu amount was recovered mainly in the cell wall-containing fraction, followed by the soluble fraction, although the mitochondria-rich fraction contained the highest specific incorporation of Cu. This accumulation pattern may cause the enhanced respiration activity that was also observed. Our small-scale proteomic analysis revealed that active energy production and consumption, as well as an increase in protein synthesis (probably in the mitochondria) and cell division contributed to root growth under Cu excess. Many
proteins known to function as Cu-binding proteins and those that are candidates for this role, including the respiratory component Complex IV, ADK, SAM, ISD, and enolase, seem to function as Cu sinks. Newly propagated root tissues, including cell walls, cytoplasm, and intracellular compartments, in addition to functional proteins, such as Cu-binding proteins, provide further space and reservoirs for additional Cu deposition.

_H. albus_ roots are tolerant to Fe deficiency and thrive under Cu excess. A comparison between this study and a previous small-scale proteomic analysis under Fe deficiency revealed that most proteins identified by the two studies differed, with the exception of 4 proteins, 3 of which show opposite behaviour in the two scenarios. Therefore, the biochemical mechanisms to tolerate Fe deficiency and to grow under Cu excess must be different. The uncharacterised protein peroxidase 27, which increased in both cases, may be used as a biomarker for the stress response. Further characterisation of peroxidase 27 may provide an insight into the mechanisms used by _H. albus_ to cope with environmental stress.

**Acknowledgements**

We wish to thank all of the members of Prof. Tatsuya Oda’s laboratory for their helpful cooperation on sample preparation for small-scale proteomics. We also thank Ms Mayumi Kudo for her technical assistance. This work was supported by a Grant-in-Aid (C, 24580479) from the Japan Society for the Promotion of Science.


Figure Legends

Fig. 1  Morphological differences between roots cultured with 0.1 and 200 μM copper (Cu).
Root tips (approximately 2.0–2.5 cm) were cultured for 2 weeks in liquid Murashige and Skoog medium. A bar indicates 1 cm.

Fig. 2  Effects of various copper (Cu) concentrations on root mass.
Root tips (approximately 2.0–2.5 cm) were cultured for 1 and 2 weeks, respectively, in liquid Murashige and Skoog medium without and with 0.1 to 200 μM Cu. n = 5. * and ** indicate significant differences at the levels of p < 0.05 and p < 0.01 compared with control (0.1 μM Cu).

Fig. 3  Comparison of respiration activity in roots cultured with 0.1 and 200 μM copper (Cu).
Respiration activity was determined using the TTC-reducing assay. n = 3. * indicates a significant difference at the levels of p < 0.05 compared with control (0.1 μM Cu).

Fig. 4  2-D gel protein profiles obtained from root tips cultured with 200 μM copper (Cu) (A) and 0.1 μM Cu (B).
Proteins (20 μg) were separated on 7-cm IPG strips (pH 5–8 linear gradient) using isoelectric focusing (IEF) in the first dimension, followed by AnykD® TGXTM gels in the second dimension. Gels were visualised with Flamingo® fluorescent staining. Differentially expressed spots were marked with arrows and numbers. The prodigy rank number was used as a spot number (see Table 5). Molecular mass (kDa) and pI are indicated on the left-hand and upper axes, respectively.
Fig. 2

Growth (mg DW/culture) vs. Cu supplied (µM) for 7-day and 14-day cultures. Bars with * indicate significant difference compared to control (0 µM Cu) at p < 0.05; bars with ** indicate significant difference at p < 0.01.
Fig. 3

The graph shows the TTC-reducing activity (μmol/h/g FW) for 0.1 μM Cu and 200 μM Cu. The data indicates a significant increase in TTC-reducing activity at 200 μM Cu compared to 0.1 μM Cu, as marked by an asterisk.
Table 1. Comparisons of lateral root developments in *Hyoscyamus albus* root cultures grown with different copper (Cu) concentrations

<table>
<thead>
<tr>
<th>Determination</th>
<th>0.1 µM Cu</th>
<th>200 µM Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of lateral roots</td>
<td>14.4 ± 2.6</td>
<td>16.2 ± 3.6</td>
</tr>
<tr>
<td>Length of lateral root (mm)</td>
<td>14.5 ± 0.7</td>
<td>18.6 ± 1.0*</td>
</tr>
<tr>
<td>Distance from the main root tip to the first lateral root (mm)</td>
<td>14.4 ± 1.2</td>
<td>9.0 ± 1.2**</td>
</tr>
</tbody>
</table>

n = 12. * and ** indicate significant differences at the levels of p < 0.05 and p < 0.01 compared with control (0.1 µM Cu).
Table 2. Copper (Cu) accumulation in *Hyoscyamus albus* roots cultured in medium supplied with various Cu concentrations

<table>
<thead>
<tr>
<th>Concentration (μM)</th>
<th>Culture period (wks)</th>
<th>µg.g⁻¹ DW</th>
<th>BF*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>2</td>
<td>17.1 ± 4.4</td>
<td>2700 ± 700</td>
</tr>
<tr>
<td>20</td>
<td>2</td>
<td>47.0 ± 0.6</td>
<td>37 ± 0.5</td>
</tr>
<tr>
<td>200</td>
<td>2</td>
<td>243.8 ± 49.5</td>
<td>19.2 ± 3.9</td>
</tr>
</tbody>
</table>

DW, dry weight. n = 3. * Bioaccumulation factor (BF) was calculated from the ratio of Cu concentration in root mass (µg. g⁻¹ DW) to that in the culture medium (µg. cm⁻³).
Table 3. Copper (Cu) distribution in various fractions from *Hyoscyamus albus* roots cultured with different Cu concentrations

<table>
<thead>
<tr>
<th>Cu supply (µM)</th>
<th>Fractionation</th>
<th>g FW/culture</th>
<th>g FW (%)</th>
<th>Total Cu (nmol)</th>
<th>Total Cu (%)</th>
<th>Cu incorporation (nmol/g FFW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>Original roots used</td>
<td>1.138 ± 0.060</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cell walls a)</td>
<td>0.395 ± 0.145</td>
<td>34.7 ± 12.7</td>
<td>12.8 ± 2.7</td>
<td>39.4 ± 8.3</td>
<td>34.4 ± 8.4</td>
</tr>
<tr>
<td></td>
<td>Nucleus and plastids b)</td>
<td>0.066 ± 0.013</td>
<td>5.8 ± 1.1</td>
<td>4.0 ± 0.7</td>
<td>12.5 ± 2.1</td>
<td>68.4 ± 14.0</td>
</tr>
<tr>
<td></td>
<td>Mitochondria c)</td>
<td>0.032 ± 0.005</td>
<td>2.8 ± 0.4</td>
<td>3.5 ± 0.5</td>
<td>10.7 ± 1.6</td>
<td>110.8 ± 27.3</td>
</tr>
<tr>
<td></td>
<td>Soluble fraction d)</td>
<td>0.645 ± 0.103</td>
<td>56.7 ± 9.1</td>
<td>12.1 ± 2.5</td>
<td>37.4 ± 7.6</td>
<td>19.4 ± 8.2</td>
</tr>
<tr>
<td>200</td>
<td>Original roots used</td>
<td>1.033 ± 0.218</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cell walls a)</td>
<td>0.517 ± 0.125</td>
<td>50.0 ± 12.1</td>
<td>279.2 ± 48.8</td>
<td>62.1 ± 10.9</td>
<td>545.5 ± 44.6</td>
</tr>
<tr>
<td></td>
<td>Nucleus and plastids b)</td>
<td>0.022 ± 0.010</td>
<td>2.1 ± 0.9</td>
<td>15.7 ± 2.8</td>
<td>3.5 ± 0.6</td>
<td>539.3 ± 115.3</td>
</tr>
<tr>
<td></td>
<td>Mitochondria c)</td>
<td>0.008 ± 0.001</td>
<td>0.8 ± 0.1</td>
<td>14.6 ± 4.6</td>
<td>3.2 ± 1.0</td>
<td>1787.5 ± 414.9</td>
</tr>
<tr>
<td></td>
<td>Soluble fraction d)</td>
<td>0.486 ± 0.082</td>
<td>47.0 ± 7.9</td>
<td>140.45 ± 28.4</td>
<td>31.2 ± 6.3</td>
<td>294.6 ± 47.2</td>
</tr>
</tbody>
</table>

a) ~ d): Original roots were fractionated into 4 fractions; cell wall-containing fraction, nucleus and plastids-rich fraction, mitochondria-rich fraction, and soluble fraction. n = 3. FFW, fraction fresh weight.
Table 4. Comparisons of various proteomics associated parameters in *Hyoscyamus albus* root culture grown with differential copper (Cu) concentrations

<table>
<thead>
<tr>
<th>Parameters</th>
<th>0.1 μM Cu</th>
<th>200 μM Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root tips (mg FW)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Protein yields (mg protein.g(^{-1})FW)</td>
<td>4.66 ± 0.37</td>
<td>5.03 ± 0.28</td>
</tr>
<tr>
<td>No. of spots detected</td>
<td>215 ± 4</td>
<td>202 ± 2</td>
</tr>
<tr>
<td>No. of spots changed (≦1.5-fold) on 2D gels*</td>
<td>34</td>
<td>(21)</td>
</tr>
<tr>
<td>(increased in relative abundance)</td>
<td></td>
<td>(13)</td>
</tr>
<tr>
<td>(decreased in relative abundance)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of protein spots identified</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>(increased in relative abundance)</td>
<td></td>
<td>(16)</td>
</tr>
<tr>
<td>(decreased in relative abundance)</td>
<td></td>
<td>(6)</td>
</tr>
</tbody>
</table>

A 20 μg volume of proteins was separated on small gels (7 cm x 7 cm). * To analyse differential protein accumulation between the two conditions of Cu availability, a threshold value (ANOVA at p < 0.05) was set at ≧1.5-fold change after normalisation using Prodigy SameSpots®.
Table 5. MS/MS-based cross-species identification and characterization of the protein spots that showed significant volume increase (upper column) and decrease (lower column), respectively, under Cu excess condition.

<table>
<thead>
<tr>
<th>Spot No.</th>
<th>Protein Name</th>
<th>Species</th>
<th>Accession No. (NCBI)</th>
<th>Theoretical Mass (kDa)/pI</th>
<th>Observed Mass (kDa)/pI</th>
<th>PSa</th>
<th>NMPa</th>
<th>SCb (%)</th>
<th>Fold (Cu1/Cu2000)</th>
<th>Subcellular Localization</th>
<th>GRAVYd</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Isocitrate dehydrogenase [NADP]</td>
<td><em>Solanum tuberosum</em></td>
<td>P50217</td>
<td>46.79/6.54</td>
<td>48.2/7.0</td>
<td>131</td>
<td>3</td>
<td>8</td>
<td>2.7</td>
<td>Cytoplasm</td>
<td>-0.307</td>
</tr>
<tr>
<td>13</td>
<td>Pyrophosphate-fructose 6-phosphate 1-phosphotransferasebeta-subunit</td>
<td><em>Solanum tuberosum</em></td>
<td>NP_001275324</td>
<td>56.49/6.21</td>
<td>62.4/7.2</td>
<td>61</td>
<td>2</td>
<td>5</td>
<td>1.8</td>
<td>Plastid or Mitochondria</td>
<td>-0.122</td>
</tr>
<tr>
<td>63</td>
<td>Enolase</td>
<td><em>Solanum lycopersicum</em></td>
<td>P26300</td>
<td>47.80/5.68</td>
<td>58.5/6.5</td>
<td>64</td>
<td>1f</td>
<td>3</td>
<td>1.6</td>
<td>Cytoplasm</td>
<td>-0.234</td>
</tr>
<tr>
<td>74</td>
<td>Pyrophosphate-fructose 6-phosphate 1-phosphotransferasebeta-subunit</td>
<td><em>Solanum tuberosum</em></td>
<td>NP_001275324</td>
<td>56.49/6.21</td>
<td>59.3/7.2</td>
<td>67</td>
<td>2</td>
<td>6</td>
<td>1.5</td>
<td>Plastid or Mitochondria</td>
<td>-0.122</td>
</tr>
<tr>
<td>17</td>
<td>Dihydrolipoamide dehydrogenase precursor</td>
<td><em>Solanum tuberosum</em></td>
<td>NP_001275339</td>
<td>53.41/6.41</td>
<td>59.3/7.0</td>
<td>77</td>
<td>1f</td>
<td>4</td>
<td>1.5</td>
<td>Mitochondria</td>
<td>0.02</td>
</tr>
</tbody>
</table>

**AMINO ACID/PROTEIN METABOLISM**

<table>
<thead>
<tr>
<th>Spot No.</th>
<th>Protein Name</th>
<th>Species</th>
<th>Accession No. (NCBI)</th>
<th>Theoretical Mass (kDa)/pI</th>
<th>Observed Mass (kDa)/pI</th>
<th>PSa</th>
<th>NMPa</th>
<th>SCb (%)</th>
<th>Fold (Cu1/Cu2000)</th>
<th>Subcellular Localization</th>
<th>GRAVYd</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Ferredoxin-nitrite reductase</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Q39161</td>
<td>65.50/5.95</td>
<td>62.4/7.0</td>
<td>70</td>
<td>2</td>
<td>4</td>
<td>2.2</td>
<td>Plastid</td>
<td>-0.396</td>
</tr>
<tr>
<td>8</td>
<td>Elongation factor Tu, mitochondrial</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Q9ZT91</td>
<td>49.41/6.25</td>
<td>45.2/7.0</td>
<td>144</td>
<td>3</td>
<td>9</td>
<td>1.7</td>
<td>Mitochondria</td>
<td>-0.178</td>
</tr>
<tr>
<td>6</td>
<td>S-adenosylmethionine synthase</td>
<td><em>Pinus banksiana</em></td>
<td>P50300</td>
<td>43.17/5.53</td>
<td>50.7/6.9</td>
<td>52</td>
<td>1f</td>
<td>4</td>
<td>1.5</td>
<td>Cytoplasm</td>
<td>-0.334</td>
</tr>
<tr>
<td>18</td>
<td>S-adenosylmethionine synthase 2</td>
<td><em>Solanum tuberosum</em></td>
<td>Q38JH8</td>
<td>42.70/5.67</td>
<td>48.2/6.8</td>
<td>102</td>
<td>2</td>
<td>7</td>
<td>1.5</td>
<td>Cytoplasm</td>
<td>-0.284</td>
</tr>
<tr>
<td>7</td>
<td>S-adenosylmethionine synthase</td>
<td><em>Brassica rapa</em></td>
<td>Q5DNB1</td>
<td>43.18/5.67</td>
<td>50.7/7.0</td>
<td>113</td>
<td>2</td>
<td>8</td>
<td>1.5</td>
<td>Cytoplasm</td>
<td>-0.334</td>
</tr>
</tbody>
</table>

**DEFENSE RESPONSE**

<table>
<thead>
<tr>
<th>Spot No.</th>
<th>Protein Name</th>
<th>Species</th>
<th>Accession No. (NCBI)</th>
<th>Theoretical Mass (kDa)/pI</th>
<th>Observed Mass (kDa)/pI</th>
<th>PSa</th>
<th>NMPa</th>
<th>SCb (%)</th>
<th>Fold (Cu1/Cu2000)</th>
<th>Subcellular Localization</th>
<th>GRAVYd</th>
</tr>
</thead>
<tbody>
<tr>
<td>1e</td>
<td>Peroxidase 27 precursor putative</td>
<td><em>Ricinus communis</em></td>
<td>XP_002280216</td>
<td>35.67/8.89</td>
<td>46.9/7.1</td>
<td>52</td>
<td>1</td>
<td>5</td>
<td>2.5</td>
<td>Secreted</td>
<td>-0.036</td>
</tr>
<tr>
<td>10</td>
<td>Heat shock cognate 70 kDa protein</td>
<td><em>Petunia x hybrida</em></td>
<td>P09189</td>
<td>71.23/5.10</td>
<td>73.9/5.2</td>
<td>141</td>
<td>3</td>
<td>7</td>
<td>1.5</td>
<td>Cytoplasm</td>
<td>-0.421</td>
</tr>
</tbody>
</table>

**ETC/ATP INVOLVED REACTION**

<table>
<thead>
<tr>
<th>Spot No.</th>
<th>Protein Name</th>
<th>Species</th>
<th>Accession No. (NCBI)</th>
<th>Theoretical Mass (kDa)/pI</th>
<th>Observed Mass (kDa)/pI</th>
<th>PSa</th>
<th>NMPa</th>
<th>SCb (%)</th>
<th>Fold (Cu1/Cu2000)</th>
<th>Subcellular Localization</th>
<th>GRAVYd</th>
</tr>
</thead>
<tbody>
<tr>
<td>57</td>
<td>Vacuolar H+-ATPase A1 subunit isoform</td>
<td><em>Solanum lycopersicum</em></td>
<td>NP_001234281</td>
<td>68.57/5.20</td>
<td>69.3/5.3</td>
<td>58</td>
<td>1f</td>
<td>7</td>
<td>2.1</td>
<td>Cytoplasm</td>
<td>-0.164</td>
</tr>
<tr>
<td>39e</td>
<td>ATP Synthase subunit alpha</td>
<td><em>Arabidopsis thaliana</em></td>
<td>P92549</td>
<td>55.05/6.23</td>
<td>57.0/6.7</td>
<td>61</td>
<td>2</td>
<td>8</td>
<td>1.5</td>
<td>Mitochondria</td>
<td>-0.029</td>
</tr>
<tr>
<td>9</td>
<td>Adenosine kinase isoform 1S</td>
<td><em>Nicotiana tabacum</em></td>
<td>AAU14832</td>
<td>37.44/5.07</td>
<td>44.6/5.1</td>
<td>49</td>
<td>1f</td>
<td>5</td>
<td>1.5</td>
<td>Cytoplasm</td>
<td>-0.187</td>
</tr>
</tbody>
</table>

**OTHERS**

<table>
<thead>
<tr>
<th>Spot No.</th>
<th>Protein Name</th>
<th>Species</th>
<th>Accession No. (NCBI)</th>
<th>Theoretical Mass (kDa)/pI</th>
<th>Observed Mass (kDa)/pI</th>
<th>PSa</th>
<th>NMPa</th>
<th>SCb (%)</th>
<th>Fold (Cu1/Cu2000)</th>
<th>Subcellular Localization</th>
<th>GRAVYd</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>Cell division control protein 48 homolog A</td>
<td><em>Arabidopsis thaliana</em></td>
<td>P54609</td>
<td>59.39/5.13</td>
<td>87.5/5.3</td>
<td>61</td>
<td>1f</td>
<td>2</td>
<td>1.8</td>
<td>Cytoplasm</td>
<td>-0.381</td>
</tr>
</tbody>
</table>

**AMINO ACID/PROTEIN METABOLISM**

<table>
<thead>
<tr>
<th>Spot No.</th>
<th>Protein Name</th>
<th>Species</th>
<th>Accession No. (NCBI)</th>
<th>Theoretical Mass (kDa)/pI</th>
<th>Observed Mass (kDa)/pI</th>
<th>PSa</th>
<th>NMPa</th>
<th>SCb (%)</th>
<th>Fold (Cu1/Cu2000)</th>
<th>Subcellular Localization</th>
<th>GRAVYd</th>
</tr>
</thead>
<tbody>
<tr>
<td>47</td>
<td>Proteasome subunit beta type-6</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Q8LD27</td>
<td>25.15/5.31</td>
<td>21.9/6.3</td>
<td>99</td>
<td>2</td>
<td>14</td>
<td>1.5</td>
<td>Cytoplasm or Nucleus</td>
<td>-0.095</td>
</tr>
<tr>
<td>72</td>
<td>Superoxide dismutase [Fe]</td>
<td><em>Nicotiana plumbaginifolia</em></td>
<td>P22302</td>
<td>23.04/5.53</td>
<td>23.6/5.3</td>
<td>58</td>
<td>2</td>
<td>9</td>
<td>1.6</td>
<td>Plastid</td>
<td>-0.392</td>
</tr>
<tr>
<td>61f</td>
<td>Glutathione peroxidase</td>
<td><em>Solanum lycopersicum</em></td>
<td>NP_001234567</td>
<td>18.84/5.76</td>
<td>18.2/6.9</td>
<td>89</td>
<td>3</td>
<td>21</td>
<td>1.5</td>
<td>Secreted</td>
<td>-0.394</td>
</tr>
</tbody>
</table>

**ETC/ATP INVOLVED REACTION**

<table>
<thead>
<tr>
<th>Spot No.</th>
<th>Protein Name</th>
<th>Species</th>
<th>Accession No. (NCBI)</th>
<th>Theoretical Mass (kDa)/pI</th>
<th>Observed Mass (kDa)/pI</th>
<th>PSa</th>
<th>NMPa</th>
<th>SCb (%)</th>
<th>Fold (Cu1/Cu2000)</th>
<th>Subcellular Localization</th>
<th>GRAVYd</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td>Predicted UMP/CMP kinase-like</td>
<td><em>Solanum lycopersicum</em></td>
<td>XP_004229700</td>
<td>22.87/5.76</td>
<td>27.2/5.4</td>
<td>83</td>
<td>2</td>
<td>14</td>
<td>1.5</td>
<td>Cytoplasm</td>
<td>-0.277</td>
</tr>
</tbody>
</table>

**OTHERS**

<table>
<thead>
<tr>
<th>Spot No.</th>
<th>Protein Name</th>
<th>Species</th>
<th>Accession No. (NCBI)</th>
<th>Theoretical Mass (kDa)/pI</th>
<th>Observed Mass (kDa)/pI</th>
<th>PSa</th>
<th>NMPa</th>
<th>SCb (%)</th>
<th>Fold (Cu1/Cu2000)</th>
<th>Subcellular Localization</th>
<th>GRAVYd</th>
</tr>
</thead>
<tbody>
<tr>
<td>49f</td>
<td>Soluble inorganic phosphatase</td>
<td><em>Solanum tuberosum</em></td>
<td>Q43187</td>
<td>24.26/5.59</td>
<td>29.0/5.7</td>
<td>58</td>
<td>1</td>
<td>8</td>
<td>1.5</td>
<td>Cytoplasm</td>
<td>-0.45</td>
</tr>
</tbody>
</table>

a Protein score (PS) and number of matched peptide (NMP) were obtained from Mascot search.

b Percentage of sequence coverage (SC) of identified peptides related to the corresponding sequence in database.

c Predicted by WOLF PSORT program using corresponding ORF (in the indicated accession number) as the query sequence.

*d GRAVY (the grand average of hydropathy) values were calculated with ProtParam tool (see Materials and Methods).

e Corresponds to the same spot position and spot number as identified by Khandakar et al. (2013).

f Annotated MS/MS spectra are provided in Supplemental data.
Spot 63. Enolase

Peptide View

MS/MS Fragmentation of **IEELGSEAVYAGASFR**
Found in **ENO_SOLLCC**, Enolase OS=Solana lycopersicum GN=PGH1 PE=2 SV=1

Match to Query 1: 1826.715724 from (1827.723000, 1+)
Title: 1: Scan 1 (rt=0, well=E1)
Data file tmpfile

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 300 to 1900 Da Full range
Label all possible matches Label matches used for scoring

Monoisotopic mass of neutral peptide Mr(calc): 1826.8686
Fixed modifications: Carboxymethyl (C) (apply to specified residues or termini only)
Ions Score: 64 Expect: 2.1e-005
Matches: 18/259 fragment ions using 30 most intense peaks (help)

<table>
<thead>
<tr>
<th>#</th>
<th>Immon.</th>
<th>a</th>
<th>a0</th>
<th>b</th>
<th>b0</th>
<th>Seq.</th>
<th>y</th>
<th>y*</th>
<th>y0</th>
<th>#</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>86.0964</td>
<td>86.0964</td>
<td>114.0913</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>102.0550</td>
<td>215.1390</td>
<td>197.1285</td>
<td>243.1339</td>
<td>225.1234</td>
<td>E</td>
<td>1714.7919</td>
<td>1697.7653</td>
<td>1696.7813</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>102.0550</td>
<td>344.1816</td>
<td>326.1710</td>
<td>372.1765</td>
<td>354.1660</td>
<td>E</td>
<td>1585.7493</td>
<td>1568.7227</td>
<td>1567.7387</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>102.0550</td>
<td>473.2242</td>
<td>455.2136</td>
<td>501.2191</td>
<td>483.2086</td>
<td>E</td>
<td>1456.7067</td>
<td>1439.6801</td>
<td>1438.6961</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>86.0964</td>
<td>586.3083</td>
<td>568.2977</td>
<td>614.3032</td>
<td>596.2926</td>
<td>L</td>
<td>1327.6641</td>
<td>1310.6375</td>
<td>1309.6535</td>
<td>13</td>
</tr>
<tr>
<td>6</td>
<td>30.3338</td>
<td>643.3297</td>
<td>625.3192</td>
<td>671.3246</td>
<td>653.3141</td>
<td>G</td>
<td>1214.5800</td>
<td>1197.5535</td>
<td>1196.5695</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>60.0441</td>
<td>730.3618</td>
<td>712.3512</td>
<td>758.3567</td>
<td>740.3461</td>
<td>S</td>
<td>1157.5586</td>
<td>1140.5320</td>
<td>1139.5480</td>
<td>11</td>
</tr>
<tr>
<td>8</td>
<td>102.0550</td>
<td>859.4044</td>
<td>841.3938</td>
<td>887.3993</td>
<td>896.3887</td>
<td>E</td>
<td>1070.5265</td>
<td>1053.5000</td>
<td>1052.5160</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>44.0495</td>
<td>930.4415</td>
<td>912.4309</td>
<td>958.4364</td>
<td>940.4258</td>
<td>A</td>
<td>941.4839</td>
<td>924.4574</td>
<td>923.4734</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>72.0808</td>
<td>1029.5099</td>
<td>1011.4993</td>
<td>1057.5048</td>
<td>1039.4942</td>
<td>V</td>
<td>870.4468</td>
<td>853.4203</td>
<td>852.4363</td>
<td>8</td>
</tr>
<tr>
<td>11</td>
<td>136.0755</td>
<td>1192.5732</td>
<td>1174.5626</td>
<td>1220.5681</td>
<td>1202.5576</td>
<td>Y</td>
<td>771.3794</td>
<td>754.3519</td>
<td>753.3679</td>
<td>7</td>
</tr>
<tr>
<td>12</td>
<td>44.0495</td>
<td>1263.6103</td>
<td>1245.5998</td>
<td>1291.6052</td>
<td>1273.5947</td>
<td>A</td>
<td>608.3151</td>
<td>591.2885</td>
<td>590.3045</td>
<td>6</td>
</tr>
<tr>
<td>13</td>
<td>30.0338</td>
<td>1320.6318</td>
<td>1302.6212</td>
<td>1348.6267</td>
<td>1330.6161</td>
<td>G</td>
<td>537.2790</td>
<td>520.2514</td>
<td>519.2674</td>
<td>5</td>
</tr>
<tr>
<td>14</td>
<td>44.0495</td>
<td>1391.6689</td>
<td>1373.6583</td>
<td>1419.6638</td>
<td>1401.6533</td>
<td>A</td>
<td>480.2565</td>
<td>463.2300</td>
<td>462.2459</td>
<td>4</td>
</tr>
<tr>
<td>15</td>
<td>60.0444</td>
<td>1478.7009</td>
<td>1460.6904</td>
<td>1506.6958</td>
<td>1488.6853</td>
<td>S</td>
<td>409.2194</td>
<td>392.1928</td>
<td>391.2088</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>120.0808</td>
<td>1625.7693</td>
<td>1607.7588</td>
<td>1653.7643</td>
<td>1635.7537</td>
<td>F</td>
<td>322.1874</td>
<td>305.1608</td>
<td>304.1768</td>
<td>2</td>
</tr>
<tr>
<td>17</td>
<td>129.1135</td>
<td>R</td>
<td></td>
<td>175.1190</td>
<td>158.0924</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>
Spot 63. Enolase (continued)

<table>
<thead>
<tr>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
</tr>
</thead>
<tbody>
<tr>
<td>EE</td>
<td>231.0975</td>
<td>259.0925</td>
<td>EEE</td>
<td>360.1401</td>
<td>388.1351</td>
<td>EEE</td>
<td>473.2242</td>
<td>501.2191</td>
</tr>
<tr>
<td>EEELG</td>
<td>330.2457</td>
<td>558.2406</td>
<td>EEEG</td>
<td>617.2777</td>
<td>645.2726</td>
<td>EE</td>
<td>231.0975</td>
<td>259.0925</td>
</tr>
<tr>
<td>EEL</td>
<td>344.1816</td>
<td>372.1765</td>
<td>EELG</td>
<td>401.2031</td>
<td>429.1980</td>
<td>EELGS</td>
<td>488.2351</td>
<td>516.2300</td>
</tr>
<tr>
<td>EELGSE</td>
<td>617.2777</td>
<td>645.2726</td>
<td>EELGSEA</td>
<td>688.3148</td>
<td>716.3097</td>
<td>EL</td>
<td>215.1390</td>
<td>243.1339</td>
</tr>
<tr>
<td>ELG</td>
<td>272.1605</td>
<td>300.1554</td>
<td>ELGS</td>
<td>359.1925</td>
<td>387.1874</td>
<td>ELGSE</td>
<td>488.2351</td>
<td>516.2300</td>
</tr>
<tr>
<td>ELGSEA</td>
<td>559.2722</td>
<td>587.2671</td>
<td>ELGSEAV</td>
<td>658.3406</td>
<td>686.3355</td>
<td>LG</td>
<td>143.1179</td>
<td>171.1128</td>
</tr>
<tr>
<td>LG</td>
<td>230.1499</td>
<td>258.1448</td>
<td>LGSE</td>
<td>359.1925</td>
<td>387.1874</td>
<td>LGSEA</td>
<td>430.2296</td>
<td>458.2245</td>
</tr>
<tr>
<td>LGSEA</td>
<td>529.2980</td>
<td>557.2930</td>
<td>LGSEAVY</td>
<td>692.3614</td>
<td>720.3563</td>
<td>GS</td>
<td>117.0659</td>
<td>145.0608</td>
</tr>
<tr>
<td>GSE</td>
<td>246.1084</td>
<td>274.1034</td>
<td>GSEA</td>
<td>317.1456</td>
<td>345.1405</td>
<td>GSEAV</td>
<td>416.2140</td>
<td>444.2089</td>
</tr>
<tr>
<td>GSEAVY</td>
<td>579.2773</td>
<td>607.2722</td>
<td>GSEAVYA</td>
<td>650.3144</td>
<td>678.3093</td>
<td>SE</td>
<td>189.0870</td>
<td>217.0819</td>
</tr>
<tr>
<td>SEA</td>
<td>260.1241</td>
<td>288.1190</td>
<td>SEA</td>
<td>359.1925</td>
<td>387.1874</td>
<td>SEAVY</td>
<td>522.2558</td>
<td>550.2508</td>
</tr>
<tr>
<td>SEAVYA</td>
<td>593.2930</td>
<td>621.2879</td>
<td>SEAVYAG</td>
<td>650.3144</td>
<td>678.3093</td>
<td>EA</td>
<td>173.0921</td>
<td>201.0870</td>
</tr>
<tr>
<td>EAV</td>
<td>272.1605</td>
<td>300.1554</td>
<td>EAVY</td>
<td>435.2238</td>
<td>463.2187</td>
<td>EAVYA</td>
<td>506.2609</td>
<td>534.2558</td>
</tr>
<tr>
<td>EAVYAG</td>
<td>563.2824</td>
<td>591.2773</td>
<td>EAVYAGA</td>
<td>634.3195</td>
<td>662.3144</td>
<td>AV</td>
<td>143.1179</td>
<td>171.1128</td>
</tr>
<tr>
<td>AV</td>
<td>366.1812</td>
<td>334.1761</td>
<td>AVYA</td>
<td>377.2183</td>
<td>405.2132</td>
<td>AVYAG</td>
<td>434.2398</td>
<td>462.2347</td>
</tr>
<tr>
<td>AVYAGA</td>
<td>505.2769</td>
<td>533.2718</td>
<td>AVYAGAS</td>
<td>592.3089</td>
<td>620.3039</td>
<td>VY</td>
<td>235.1441</td>
<td>263.1390</td>
</tr>
<tr>
<td>VY</td>
<td>366.1812</td>
<td>334.1761</td>
<td>VYAG</td>
<td>363.2027</td>
<td>391.1976</td>
<td>VYAGA</td>
<td>434.2398</td>
<td>462.2347</td>
</tr>
<tr>
<td>VYAGAS</td>
<td>521.2718</td>
<td>549.2667</td>
<td>VYAGASF</td>
<td>668.3402</td>
<td>696.3352</td>
<td>YA</td>
<td>207.1128</td>
<td>235.1077</td>
</tr>
<tr>
<td>YAG</td>
<td>264.1343</td>
<td>292.1292</td>
<td>YAGA</td>
<td>335.1714</td>
<td>363.1663</td>
<td>YAGAS</td>
<td>422.2034</td>
<td>450.1983</td>
</tr>
<tr>
<td>YAGASF</td>
<td>569.2718</td>
<td>597.2667</td>
<td>AG</td>
<td>101.0709</td>
<td>129.0659</td>
<td>AGA</td>
<td>172.1081</td>
<td>200.1030</td>
</tr>
<tr>
<td>AGAS</td>
<td>259.1401</td>
<td>287.1350</td>
<td>AGASF</td>
<td>406.2085</td>
<td>434.2034</td>
<td>GA</td>
<td>101.0709</td>
<td>129.0659</td>
</tr>
<tr>
<td>GAS</td>
<td>188.1030</td>
<td>216.0979</td>
<td>GASF</td>
<td>335.1714</td>
<td>363.1663</td>
<td>AS</td>
<td>131.0815</td>
<td>159.0764</td>
</tr>
<tr>
<td>ASF</td>
<td>278.1499</td>
<td>306.1448</td>
<td>SF</td>
<td>207.1128</td>
<td>235.1077</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Spot 67. Fructose-bisphosphate aldolase-like protein

Peptide View

MS/MS Fragmentation of **IGANEPSQALINENANGLAR**
Found in gil25014723, KS09020F02 KS09 Capsicum annum cDNA, mRNA sequence
Translated in frame 3 (nucleic acid sequence)

Match to Query 1: 2051.272724 from(2052.280000,1+)
Title: 1 Scan (rt=0, well=F2)
Data file tempfile

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from [400] to [2100] Da [Full range]
Label all possible matches [Label matches used for scoring]

Monoisotopic mass of neutral peptide Mr(calc): 2051.0395
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 55 Expect: 0.0095
Matches : 14/329 fragment ions using 14 most intense peaks (help)

<table>
<thead>
<tr>
<th>#</th>
<th>Immon.</th>
<th>a</th>
<th>a*</th>
<th>a0</th>
<th>b</th>
<th>b*</th>
<th>b0</th>
<th>Seq.</th>
<th>y</th>
<th>y*</th>
<th>y0</th>
<th>#</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>86.0964</td>
<td>86.0964</td>
<td>114.0913</td>
<td>I</td>
<td>1938.9628</td>
<td>1921.9362</td>
<td>1920.9522</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>30.0338</td>
<td>143.1179</td>
<td>171.1128</td>
<td>G</td>
<td>1881.9413</td>
<td>1864.9148</td>
<td>1863.9308</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>44.0495</td>
<td>214.1550</td>
<td>242.1499</td>
<td>A</td>
<td>1810.9042</td>
<td>1793.8777</td>
<td>1792.8936</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>87.0553</td>
<td>328.1979</td>
<td>356.1928</td>
<td>339.1663</td>
<td>N</td>
<td>1696.8613</td>
<td>1679.8347</td>
<td>1678.8507</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>102.0550</td>
<td>457.2405</td>
<td>485.2354</td>
<td>468.2089</td>
<td>E</td>
<td>1567.8187</td>
<td>1550.7921</td>
<td>1549.8081</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>70.0651</td>
<td>554.2933</td>
<td>582.2882</td>
<td>P</td>
<td>1527.7554</td>
<td>1501.7288</td>
<td>1499.7444</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>60.0444</td>
<td>641.3253</td>
<td>659.3882</td>
<td>642.3505</td>
<td>S</td>
<td>1470.7659</td>
<td>1453.7394</td>
<td>1452.7554</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>101.0709</td>
<td>769.3839</td>
<td>797.3788</td>
<td>780.3523</td>
<td>Q</td>
<td>1393.7339</td>
<td>1366.7074</td>
<td>1365.7233</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>86.0964</td>
<td>882.4680</td>
<td>900.4629</td>
<td>893.4363</td>
<td>L</td>
<td>1255.6753</td>
<td>1238.6488</td>
<td>1237.6648</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>44.0495</td>
<td>953.5051</td>
<td>981.4980</td>
<td>964.4734</td>
<td>A</td>
<td>1142.5913</td>
<td>1125.5647</td>
<td>1124.5807</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>86.0964</td>
<td>1066.5891</td>
<td>1094.5840</td>
<td>1077.5575</td>
<td>I</td>
<td>1071.5541</td>
<td>1054.5276</td>
<td>1053.5436</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>87.0553</td>
<td>1180.6321</td>
<td>1208.6270</td>
<td>1191.6004</td>
<td>N</td>
<td>958.4703</td>
<td>941.4431</td>
<td>940.4595</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>102.0550</td>
<td>1309.6747</td>
<td>1337.6696</td>
<td>1320.6430</td>
<td>E</td>
<td>844.4272</td>
<td>827.4006</td>
<td>826.4168</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>87.0553</td>
<td>1423.7176</td>
<td>1451.7125</td>
<td>1434.6859</td>
<td>N</td>
<td>717.3845</td>
<td>698.3580</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>44.0495</td>
<td>1494.7547</td>
<td>1522.7495</td>
<td>1505.7231</td>
<td>A</td>
<td>601.3416</td>
<td>584.3151</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>87.0553</td>
<td>1608.7976</td>
<td>1636.7925</td>
<td>1619.7660</td>
<td>N</td>
<td>530.3048</td>
<td>513.2780</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>30.0338</td>
<td>1665.8191</td>
<td>1693.8140</td>
<td>1676.7875</td>
<td>G</td>
<td>416.2616</td>
<td>399.2350</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>86.0964</td>
<td>1778.9032</td>
<td>1806.8981</td>
<td>1789.8715</td>
<td>L</td>
<td>359.2401</td>
<td>342.2136</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>44.0495</td>
<td>1849.9403</td>
<td>1877.9352</td>
<td>1860.9086</td>
<td>A</td>
<td>246.1561</td>
<td>229.1295</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>129.1135</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>175.1190</td>
<td>158.0924</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Spot 67. Fructose-bisphosphate aldolase–like protein (continued)

<table>
<thead>
<tr>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA</td>
<td>101.0709</td>
<td>129.0659</td>
<td>GAN</td>
<td>215.1139</td>
<td>243.1088</td>
<td>GANE</td>
<td>344.1565</td>
<td>372.1514</td>
</tr>
<tr>
<td>GANE</td>
<td>441.2092</td>
<td>469.2041</td>
<td>GANEPS</td>
<td>528.2413</td>
<td>556.2362</td>
<td>GANEPSQ</td>
<td>656.2998</td>
<td>684.2947</td>
</tr>
<tr>
<td>AN</td>
<td>158.0924</td>
<td>186.0873</td>
<td>ANE</td>
<td>287.1350</td>
<td>315.1299</td>
<td>ANEP</td>
<td>384.1878</td>
<td>412.1827</td>
</tr>
<tr>
<td>ANEPS</td>
<td>471.2198</td>
<td>499.2147</td>
<td>ANEPSQ</td>
<td>599.2784</td>
<td>627.2733</td>
<td>NE</td>
<td>216.0979</td>
<td>244.0928</td>
</tr>
<tr>
<td>NE</td>
<td>313.1506</td>
<td>341.1456</td>
<td>NEPS</td>
<td>400.1827</td>
<td>428.1776</td>
<td>NEPSQ</td>
<td>528.2413</td>
<td>556.2362</td>
</tr>
<tr>
<td>NEPSQ</td>
<td>641.3253</td>
<td>669.3202</td>
<td>EPS</td>
<td>199.1077</td>
<td>227.1026</td>
<td>EPSQ</td>
<td>286.1397</td>
<td>314.1347</td>
</tr>
<tr>
<td>EPSQ</td>
<td>414.1983</td>
<td>442.1932</td>
<td>EPSQL</td>
<td>527.2824</td>
<td>555.2773</td>
<td>EPSQL</td>
<td>598.3195</td>
<td>626.3144</td>
</tr>
<tr>
<td>PS</td>
<td>157.0972</td>
<td>185.0921</td>
<td>PSQ</td>
<td>285.1557</td>
<td>313.1506</td>
<td>PSQL</td>
<td>398.2398</td>
<td>426.2347</td>
</tr>
<tr>
<td>PSQ</td>
<td>469.2769</td>
<td>497.2718</td>
<td>PSQLAI</td>
<td>582.3610</td>
<td>610.3559</td>
<td>PSQLAIN</td>
<td>696.4039</td>
<td>724.3988</td>
</tr>
<tr>
<td>SQ</td>
<td>188.1030</td>
<td>216.0979</td>
<td>SQAL</td>
<td>301.1870</td>
<td>329.1819</td>
<td>SQAL</td>
<td>372.2241</td>
<td>400.2191</td>
</tr>
<tr>
<td>SQLAI</td>
<td>485.3082</td>
<td>513.3031</td>
<td>SQLAIN</td>
<td>599.3511</td>
<td>627.3461</td>
<td>QL</td>
<td>214.1550</td>
<td>242.1499</td>
</tr>
<tr>
<td>QL</td>
<td>285.1921</td>
<td>313.1870</td>
<td>QLAI</td>
<td>398.2762</td>
<td>426.2711</td>
<td>QLAIN</td>
<td>512.3191</td>
<td>540.3140</td>
</tr>
<tr>
<td>QLAI</td>
<td>641.3617</td>
<td>669.3566</td>
<td>LA</td>
<td>157.1335</td>
<td>185.1285</td>
<td>LAIN</td>
<td>270.2176</td>
<td>298.2125</td>
</tr>
<tr>
<td>LAIN</td>
<td>384.2605</td>
<td>412.2554</td>
<td>LAINE</td>
<td>513.3031</td>
<td>541.2980</td>
<td>LAINEN</td>
<td>627.3460</td>
<td>655.3410</td>
</tr>
<tr>
<td>LAINEN</td>
<td>698.3832</td>
<td>726.3781</td>
<td>AI</td>
<td>157.1335</td>
<td>185.1285</td>
<td>AIN</td>
<td>271.1765</td>
<td>299.1714</td>
</tr>
<tr>
<td>AINE</td>
<td>400.2191</td>
<td>428.2140</td>
<td>AINEN</td>
<td>514.2620</td>
<td>542.2569</td>
<td>AINEA</td>
<td>585.2991</td>
<td>613.2940</td>
</tr>
<tr>
<td>AINEA</td>
<td>699.3420</td>
<td>727.3369</td>
<td>IN</td>
<td>200.1394</td>
<td>228.1343</td>
<td>INE</td>
<td>329.1819</td>
<td>357.1769</td>
</tr>
<tr>
<td>IN</td>
<td>443.2249</td>
<td>471.2198</td>
<td>INENA</td>
<td>514.2620</td>
<td>542.2569</td>
<td>INENAN</td>
<td>628.3049</td>
<td>656.2998</td>
</tr>
<tr>
<td>INENAN</td>
<td>685.3264</td>
<td>713.3213</td>
<td>NE</td>
<td>216.0979</td>
<td>244.0928</td>
<td>NEN</td>
<td>330.1408</td>
<td>358.1357</td>
</tr>
<tr>
<td>NEN</td>
<td>401.1779</td>
<td>429.1728</td>
<td>NENAN</td>
<td>515.2208</td>
<td>543.2158</td>
<td>NENANG</td>
<td>572.2423</td>
<td>600.2372</td>
</tr>
<tr>
<td>NENANG</td>
<td>685.3264</td>
<td>713.3213</td>
<td>EN</td>
<td>216.0979</td>
<td>244.0928</td>
<td>ENA</td>
<td>287.1350</td>
<td>315.1299</td>
</tr>
<tr>
<td>EN</td>
<td>401.1779</td>
<td>429.1728</td>
<td>ENANG</td>
<td>458.1994</td>
<td>486.1943</td>
<td>ENANGL</td>
<td>571.2834</td>
<td>599.2784</td>
</tr>
<tr>
<td>ENANGL</td>
<td>642.3206</td>
<td>670.3155</td>
<td>NA</td>
<td>158.0924</td>
<td>186.0873</td>
<td>NAN</td>
<td>272.1353</td>
<td>300.1302</td>
</tr>
<tr>
<td>NANG</td>
<td>329.1568</td>
<td>357.1517</td>
<td>NANGL</td>
<td>442.2409</td>
<td>470.2358</td>
<td>NANGL</td>
<td>513.2780</td>
<td>541.2729</td>
</tr>
<tr>
<td>ANG</td>
<td>158.0924</td>
<td>186.0873</td>
<td>ANGL</td>
<td>215.1139</td>
<td>243.1088</td>
<td>ANGL</td>
<td>328.1979</td>
<td>356.1928</td>
</tr>
<tr>
<td>ANGL</td>
<td>399.2350</td>
<td>427.2300</td>
<td>NG</td>
<td>144.0768</td>
<td>172.0717</td>
<td>NGL</td>
<td>257.1608</td>
<td>285.1557</td>
</tr>
<tr>
<td>NGL</td>
<td>328.1979</td>
<td>356.1928</td>
<td>GL</td>
<td>143.1179</td>
<td>171.1128</td>
<td>GLA</td>
<td>214.1550</td>
<td>242.1499</td>
</tr>
</tbody>
</table>

**Graphs:**
- **Left:** Error vs. Mass (Da) with R2 = 0.9272.
- **Right:** Error vs. Mass (Da) with R2 = 0.9272.
Spot 17. Dihydrolipoamide dehydrogenase precursor

Peptide View

MS/MS Fragmentation of FLSPSEISVDTVEGGNSVVK
Found in gi|8894612, EST282165 tomato callus, TAMU Solanum lycopersicum cDNA clone eLEC36P6, mRNA sequence
Translated in frame 2 (nucleic acid sequence)

Match to Query 1: 2063.347724 from(2064.355000,1+)
Title: 1 Scan 1 (rt=0, well=F9)
Data file tempfile

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 300 to 2100 Da  Full range
Label all possible matches  Label matches used for scoring

Monoisotopic mass of neutral peptide Mr(calc): 2063.0423
Fixed modifications: Carboxymethyl (C) (apply to specified residues or termini only)
Ions Score: 77  Expect: 7.4e-005
Matches : 14/318 fragment ions using 17 most intense peaks  (help)

<table>
<thead>
<tr>
<th>#</th>
<th>Immon.</th>
<th>a</th>
<th>a*</th>
<th>a0</th>
<th>b</th>
<th>b*</th>
<th>h0</th>
<th>Seq.</th>
<th>y</th>
<th>y*</th>
<th>y0</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>120.0808</td>
<td>120.0808</td>
<td></td>
<td></td>
<td>148.0757</td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>86.0964</td>
<td>233.1648</td>
<td></td>
<td></td>
<td>261.1598</td>
<td></td>
<td></td>
<td>L</td>
<td>1916.9811</td>
<td>1899.9546</td>
<td>1898.9706</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>60.0444</td>
<td>320.1969</td>
<td>302.1863</td>
<td>348.1918</td>
<td>330.1812</td>
<td></td>
<td></td>
<td>S</td>
<td>1803.8971</td>
<td>1786.8705</td>
<td>1785.8865</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>70.0651</td>
<td>417.2496</td>
<td>399.2391</td>
<td>445.2445</td>
<td>427.2340</td>
<td></td>
<td></td>
<td>P</td>
<td>1716.8650</td>
<td>1699.8385</td>
<td>1698.8545</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>60.0444</td>
<td>504.2817</td>
<td>486.2711</td>
<td>532.2766</td>
<td>514.2660</td>
<td></td>
<td></td>
<td>S</td>
<td>1619.8123</td>
<td>1602.7857</td>
<td>1601.8017</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>102.0550</td>
<td>633.3243</td>
<td>615.3137</td>
<td>661.3192</td>
<td>643.3086</td>
<td></td>
<td></td>
<td>E</td>
<td>1532.7802</td>
<td>1515.7537</td>
<td>1514.7697</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>86.0964</td>
<td>746.4083</td>
<td>728.3978</td>
<td>774.4032</td>
<td>756.3927</td>
<td></td>
<td></td>
<td>I</td>
<td>1403.7377</td>
<td>1386.7111</td>
<td>1385.7271</td>
<td>14</td>
</tr>
<tr>
<td>8</td>
<td>60.0444</td>
<td>833.4403</td>
<td>815.4298</td>
<td>861.4353</td>
<td>843.4247</td>
<td></td>
<td></td>
<td>S</td>
<td>1290.6536</td>
<td>1273.6270</td>
<td>1272.6430</td>
<td>13</td>
</tr>
<tr>
<td>9</td>
<td>72.0808</td>
<td>932.5088</td>
<td>914.4982</td>
<td>960.5037</td>
<td>942.4931</td>
<td></td>
<td></td>
<td>V</td>
<td>1203.6216</td>
<td>1186.5950</td>
<td>1185.6110</td>
<td>12</td>
</tr>
<tr>
<td>10</td>
<td>88.0393</td>
<td>1047.5357</td>
<td>1029.5251</td>
<td>1075.5306</td>
<td>1057.5201</td>
<td>D</td>
<td></td>
<td>1104.5531</td>
<td>1087.5266</td>
<td>1086.5426</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>74.0600</td>
<td>1148.5834</td>
<td>1130.5728</td>
<td>1176.5783</td>
<td>1158.5677</td>
<td>T</td>
<td></td>
<td>989.5262</td>
<td>972.4997</td>
<td>971.5156</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>72.0808</td>
<td>1247.6518</td>
<td>1229.6412</td>
<td>1275.6467</td>
<td>1257.6361</td>
<td>V</td>
<td></td>
<td>888.4785</td>
<td>871.4520</td>
<td>870.4680</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>102.0550</td>
<td>1376.6944</td>
<td>1358.6838</td>
<td>1404.6893</td>
<td>1385.6787</td>
<td>E</td>
<td></td>
<td>799.4101</td>
<td>772.3836</td>
<td>771.3995</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>30.0338</td>
<td>1433.7159</td>
<td>1415.7053</td>
<td>1461.7108</td>
<td>1443.7002</td>
<td>G</td>
<td></td>
<td>660.3675</td>
<td>643.3410</td>
<td>642.3570</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>30.0338</td>
<td>1490.7373</td>
<td>1472.7267</td>
<td>1518.7322</td>
<td>1500.7217</td>
<td>G</td>
<td></td>
<td>603.3461</td>
<td>586.3195</td>
<td>585.3355</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>87.0553</td>
<td>1604.7802</td>
<td>1587.7537</td>
<td>1636.7679</td>
<td>1632.7752</td>
<td>1615.7486</td>
<td>1614.7646</td>
<td>N</td>
<td>546.3246</td>
<td>529.2980</td>
<td>528.3140</td>
<td>5</td>
</tr>
<tr>
<td>17</td>
<td>60.0444</td>
<td>1691.8123</td>
<td>1674.7875</td>
<td>1719.8017</td>
<td>1719.8072</td>
<td>1702.7806</td>
<td>1701.7966</td>
<td>S</td>
<td>432.2817</td>
<td>415.2551</td>
<td>414.2711</td>
<td>4</td>
</tr>
<tr>
<td>18</td>
<td>72.0808</td>
<td>1790.8807</td>
<td>1773.8541</td>
<td>1817.8701</td>
<td>1818.8756</td>
<td>1801.8491</td>
<td>1800.8650</td>
<td>V</td>
<td>345.2496</td>
<td>328.2231</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>19</td>
<td>72.0808</td>
<td>1889.9491</td>
<td>1872.9225</td>
<td>1871.9385</td>
<td>1917.9440</td>
<td>1900.9175</td>
<td>1899.9334</td>
<td>V</td>
<td>246.1812</td>
<td>229.1547</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>20</td>
<td>101.1073</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>K</td>
<td>147.1128</td>
<td>130.0863</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>
### Spot 17. Dihydrolipoamide dehydrogenase precursor (continued)

<table>
<thead>
<tr>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS</td>
<td>173.1285</td>
<td>201.1234</td>
<td>LSP</td>
<td>270.1812</td>
<td>298.1761</td>
<td>LSPS</td>
<td>357.2132</td>
<td>385.2082</td>
</tr>
<tr>
<td>LSPSE</td>
<td>486.2558</td>
<td>514.2508</td>
<td>LSPSEI</td>
<td>599.3399</td>
<td>627.3348</td>
<td>LSPSEIS</td>
<td>686.3719</td>
<td>714.3668</td>
</tr>
<tr>
<td>SP</td>
<td>157.0972</td>
<td>185.0921</td>
<td>SPS</td>
<td>244.1292</td>
<td>272.1241</td>
<td>SPSE</td>
<td>373.1718</td>
<td>401.1667</td>
</tr>
<tr>
<td>SPSEI</td>
<td>486.2558</td>
<td>514.2508</td>
<td>SPSEIS</td>
<td>573.2879</td>
<td>601.2828</td>
<td>SPSEISV</td>
<td>672.3563</td>
<td>700.3512</td>
</tr>
<tr>
<td>PS</td>
<td>157.0972</td>
<td>185.0921</td>
<td>PSE</td>
<td>286.1397</td>
<td>314.1347</td>
<td>PSEI</td>
<td>399.2238</td>
<td>427.2187</td>
</tr>
<tr>
<td>PSEIS</td>
<td>486.2558</td>
<td>514.2508</td>
<td>PSEISV</td>
<td>585.3243</td>
<td>613.3192</td>
<td>SE</td>
<td>189.0870</td>
<td>217.0819</td>
</tr>
<tr>
<td>SE</td>
<td>302.1710</td>
<td>330.1660</td>
<td>SEIS</td>
<td>389.2031</td>
<td>417.1980</td>
<td>SEISV</td>
<td>488.2715</td>
<td>516.2664</td>
</tr>
<tr>
<td>SEISV</td>
<td>603.2984</td>
<td>631.2933</td>
<td>EI</td>
<td>215.1390</td>
<td>243.1339</td>
<td>EIS</td>
<td>302.1710</td>
<td>330.1660</td>
</tr>
<tr>
<td>EISV</td>
<td>401.2395</td>
<td>429.2344</td>
<td>EISVD</td>
<td>516.2664</td>
<td>544.2613</td>
<td>EISVDT</td>
<td>617.3141</td>
<td>645.3090</td>
</tr>
<tr>
<td>IS</td>
<td>173.1285</td>
<td>201.1234</td>
<td>ISV</td>
<td>272.1969</td>
<td>300.1918</td>
<td>ISVD</td>
<td>387.2238</td>
<td>415.2187</td>
</tr>
<tr>
<td>ISVD</td>
<td>488.2715</td>
<td>516.2664</td>
<td>ISVDT</td>
<td>587.3399</td>
<td>615.3348</td>
<td>SV</td>
<td>159.1128</td>
<td>187.1077</td>
</tr>
<tr>
<td>SVD</td>
<td>274.1397</td>
<td>302.1347</td>
<td>SVDT</td>
<td>375.1874</td>
<td>403.1823</td>
<td>SVDTV</td>
<td>474.2558</td>
<td>502.2508</td>
</tr>
<tr>
<td>SVDTVE</td>
<td>603.2984</td>
<td>631.2933</td>
<td>SVDTVEG</td>
<td>660.3199</td>
<td>688.3148</td>
<td>VD</td>
<td>187.1077</td>
<td>215.1026</td>
</tr>
<tr>
<td>VDT</td>
<td>288.1554</td>
<td>316.1503</td>
<td>VDTV</td>
<td>387.2238</td>
<td>415.2187</td>
<td>VDTVE</td>
<td>516.2664</td>
<td>544.2613</td>
</tr>
<tr>
<td>VDTVEG</td>
<td>573.2879</td>
<td>601.2828</td>
<td>VDTVEGG</td>
<td>630.3093</td>
<td>658.3042</td>
<td>DT</td>
<td>189.0870</td>
<td>217.0819</td>
</tr>
<tr>
<td>DTVEGG</td>
<td>531.2409</td>
<td>559.2358</td>
<td>DTVEGGN</td>
<td>645.2838</td>
<td>673.2788</td>
<td>TV</td>
<td>173.1285</td>
<td>201.1234</td>
</tr>
<tr>
<td>TVE</td>
<td>302.1710</td>
<td>330.1660</td>
<td>TVEG</td>
<td>359.1925</td>
<td>387.1874</td>
<td>TVEGG</td>
<td>416.2140</td>
<td>444.2089</td>
</tr>
<tr>
<td>TVEGGN</td>
<td>530.2569</td>
<td>558.2518</td>
<td>TVEGGNS</td>
<td>617.2889</td>
<td>645.2838</td>
<td>VE</td>
<td>201.1234</td>
<td>229.1183</td>
</tr>
<tr>
<td>VEG</td>
<td>258.1448</td>
<td>286.1397</td>
<td>VEGG</td>
<td>315.1663</td>
<td>343.1612</td>
<td>VEGGN</td>
<td>429.2092</td>
<td>457.2041</td>
</tr>
<tr>
<td>VEGGS</td>
<td>516.2413</td>
<td>544.2362</td>
<td>VEGGSV</td>
<td>615.3097</td>
<td>643.3046</td>
<td>EG</td>
<td>159.0764</td>
<td>187.0713</td>
</tr>
<tr>
<td>EGG</td>
<td>216.0979</td>
<td>244.0929</td>
<td>EGGN</td>
<td>330.1408</td>
<td>358.1357</td>
<td>EGGNS</td>
<td>417.1728</td>
<td>445.1678</td>
</tr>
<tr>
<td>EGGNSV</td>
<td>516.2413</td>
<td>544.2362</td>
<td>EGGNSVV</td>
<td>615.3097</td>
<td>643.3046</td>
<td>GG</td>
<td>87.0553</td>
<td>115.0502</td>
</tr>
<tr>
<td>GGN</td>
<td>201.0982</td>
<td>229.0931</td>
<td>GGNS</td>
<td>288.1302</td>
<td>316.1252</td>
<td>GGNSV</td>
<td>387.1987</td>
<td>415.1936</td>
</tr>
<tr>
<td>GGNSV</td>
<td>486.2671</td>
<td>514.2620</td>
<td>GN</td>
<td>144.0768</td>
<td>172.0717</td>
<td>GNS</td>
<td>231.1088</td>
<td>259.1037</td>
</tr>
<tr>
<td>GNSV</td>
<td>330.1772</td>
<td>358.1721</td>
<td>GNSVV</td>
<td>429.2456</td>
<td>457.2405</td>
<td>NS</td>
<td>174.0837</td>
<td>202.0822</td>
</tr>
<tr>
<td>NSV</td>
<td>273.1557</td>
<td>301.1506</td>
<td>NSVV</td>
<td>372.2241</td>
<td>400.2191</td>
<td>SV</td>
<td>159.1128</td>
<td>187.1077</td>
</tr>
<tr>
<td>SVV</td>
<td>258.1812</td>
<td>286.1761</td>
<td>VV</td>
<td>171.1492</td>
<td>199.1441</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Spot 6. S-adenosylmethionine synthase

Peptide View

MS/MS Fragmentation of YLDENTIFHLNPSGR
Found in METK_PINBN, S-adenosylmethionine synthase OS=Pinus banksiana GN=METK PE=2 SV=1

Match to Query 1: 1774.462724 from(1775.470000,1+)
Title: 1.Scan 1 (rt=0, well=A12)
Data file tempfile

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 300 to 1800 Da Full range
Label all possible matches Label matches used for scoring

Monoisotopic mass of neutral peptide Mr(calc): 1774.8638
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 52 Expect: 0.00034
Matches : 17/221 fragment ions using 28 most intense peaks (help)

<table>
<thead>
<tr>
<th>#</th>
<th>Imonon.</th>
<th>a</th>
<th>a°</th>
<th>a*</th>
<th>b</th>
<th>b°</th>
<th>b*</th>
<th>Seq.</th>
<th>y</th>
<th>y°</th>
<th>y*</th>
<th>#</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>136.0757</td>
<td>136.0757</td>
<td></td>
<td>164.0706</td>
<td></td>
<td></td>
<td></td>
<td>Y</td>
<td>1612.8078</td>
<td>1595.7812</td>
<td>1594.7972</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>86.0964</td>
<td>249.1598</td>
<td></td>
<td>277.1547</td>
<td></td>
<td></td>
<td></td>
<td>L</td>
<td>1499.7237</td>
<td>1482.6972</td>
<td>1481.7132</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>88.0393</td>
<td>364.1867</td>
<td></td>
<td>346.1761</td>
<td>392.1816</td>
<td></td>
<td>374.1710</td>
<td>D</td>
<td>1384.6968</td>
<td>1367.6702</td>
<td>1366.6862</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>102.0550</td>
<td>493.2293</td>
<td></td>
<td>475.2187</td>
<td>521.2242</td>
<td></td>
<td>503.2136</td>
<td>E</td>
<td>1141.6113</td>
<td>1124.5847</td>
<td>1123.6007</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>87.0553</td>
<td>607.2722</td>
<td></td>
<td>590.2457</td>
<td>589.2617</td>
<td>635.2671</td>
<td>618.2406</td>
<td>N</td>
<td>1040.5636</td>
<td>1023.5370</td>
<td>1022.5530</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>74.0600</td>
<td>708.3199</td>
<td></td>
<td>691.2933</td>
<td>690.3093</td>
<td>736.3148</td>
<td>719.2883</td>
<td>T</td>
<td>927.4795</td>
<td>910.4530</td>
<td>909.4690</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>86.0964</td>
<td>821.4040</td>
<td></td>
<td>804.3774</td>
<td>803.3934</td>
<td>849.3899</td>
<td>832.3723</td>
<td>I</td>
<td>870.4111</td>
<td>763.3846</td>
<td>762.4005</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>120.0808</td>
<td>968.4724</td>
<td>951.4458</td>
<td>950.4618</td>
<td>996.4673</td>
<td>979.4407</td>
<td>978.4567</td>
<td>F</td>
<td>643.3522</td>
<td>626.3256</td>
<td>625.3416</td>
<td>6</td>
</tr>
<tr>
<td>9</td>
<td>110.0713</td>
<td>1105.5313</td>
<td>1088.5047</td>
<td>1087.5207</td>
<td>1133.5262</td>
<td>1116.4997</td>
<td>1115.5156</td>
<td>H</td>
<td>530.2681</td>
<td>513.2416</td>
<td>512.2576</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>86.0964</td>
<td>1218.6154</td>
<td>1201.5888</td>
<td>1200.6048</td>
<td>1246.6103</td>
<td>1229.5837</td>
<td>1228.5997</td>
<td>L</td>
<td>416.2252</td>
<td>399.1987</td>
<td>398.2146</td>
<td>4</td>
</tr>
<tr>
<td>11</td>
<td>87.0553</td>
<td>1332.6583</td>
<td>1315.6317</td>
<td>1314.6477</td>
<td>1360.6532</td>
<td>1343.6266</td>
<td>1342.6426</td>
<td>N</td>
<td>319.1724</td>
<td>302.1459</td>
<td>301.1619</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>70.0651</td>
<td>1429.7110</td>
<td>1412.6845</td>
<td>1411.7005</td>
<td>1457.7060</td>
<td>1440.6794</td>
<td>1439.6954</td>
<td>P</td>
<td>232.1404</td>
<td>215.1139</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>60.0444</td>
<td>1516.7431</td>
<td>1499.7165</td>
<td>1498.7325</td>
<td>1544.7380</td>
<td>1527.7114</td>
<td>1526.7274</td>
<td>S</td>
<td>175.1190</td>
<td>158.0924</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>30.0338</td>
<td>1573.7645</td>
<td>1556.7380</td>
<td>1555.7540</td>
<td>1601.7594</td>
<td>1584.7329</td>
<td>1583.7489</td>
<td>G</td>
<td>302.1459</td>
<td>301.1619</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>129.1135</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R</td>
<td>158.0924</td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>
Spot 6. S-adenosylmethionine synthase (continued)

<table>
<thead>
<tr>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD</td>
<td>201.1234</td>
<td>229.1183</td>
<td>LDEN</td>
<td>330.1660</td>
<td>358.1609</td>
<td>LDEN</td>
<td>444.2089</td>
<td>472.2038</td>
</tr>
<tr>
<td>LDENT</td>
<td>545.2566</td>
<td>573.2515</td>
<td>LDENTI</td>
<td>658.3406</td>
<td>686.3355</td>
<td>DE</td>
<td>217.0819</td>
<td>245.0768</td>
</tr>
<tr>
<td>DEN</td>
<td>331.1248</td>
<td>359.1197</td>
<td>DENT</td>
<td>432.1725</td>
<td>460.1674</td>
<td>DENTI</td>
<td>545.2566</td>
<td>573.2515</td>
</tr>
<tr>
<td>DENTIF</td>
<td>692.3250</td>
<td>720.3199</td>
<td>EN</td>
<td>216.6979</td>
<td>244.0928</td>
<td>ENT</td>
<td>317.1456</td>
<td>345.1405</td>
</tr>
<tr>
<td>ENTI</td>
<td>430.2296</td>
<td>458.2245</td>
<td>ENTIIF</td>
<td>577.2980</td>
<td>605.2930</td>
<td>NT</td>
<td>188.1030</td>
<td>216.0979</td>
</tr>
<tr>
<td>NTI</td>
<td>301.1870</td>
<td>329.1819</td>
<td>NTIF</td>
<td>448.2554</td>
<td>476.2504</td>
<td>NTIFH</td>
<td>585.3144</td>
<td>613.3093</td>
</tr>
<tr>
<td>NTIFH</td>
<td>698.3984</td>
<td>726.3933</td>
<td>TI</td>
<td>187.1441</td>
<td>215.1390</td>
<td>TIF</td>
<td>334.2125</td>
<td>362.2074</td>
</tr>
<tr>
<td>TIFH</td>
<td>471.2714</td>
<td>499.2663</td>
<td>TIFHL</td>
<td>584.3355</td>
<td>612.3304</td>
<td>TIFHLN</td>
<td>698.3984</td>
<td>726.3933</td>
</tr>
<tr>
<td>IF</td>
<td>233.1648</td>
<td>261.1598</td>
<td>IFH</td>
<td>370.2238</td>
<td>398.2187</td>
<td>IFHL</td>
<td>483.3078</td>
<td>511.3027</td>
</tr>
<tr>
<td>IFHLN</td>
<td>597.3507</td>
<td>625.3457</td>
<td>IFHLNP</td>
<td>694.4035</td>
<td>722.3984</td>
<td>FH</td>
<td>257.1397</td>
<td>285.1346</td>
</tr>
<tr>
<td>FIHL</td>
<td>370.2238</td>
<td>398.2187</td>
<td>FIHLN</td>
<td>484.2667</td>
<td>512.2616</td>
<td>FIHLNP</td>
<td>581.3194</td>
<td>609.3144</td>
</tr>
<tr>
<td>HLN</td>
<td>434.2510</td>
<td>462.2459</td>
<td>HLNPS</td>
<td>521.2831</td>
<td>549.2780</td>
<td>HLNPSG</td>
<td>578.3045</td>
<td>606.2994</td>
</tr>
<tr>
<td>LN</td>
<td>200.1394</td>
<td>228.1343</td>
<td>LNP</td>
<td>297.1921</td>
<td>325.1870</td>
<td>LNPS</td>
<td>384.2241</td>
<td>412.2191</td>
</tr>
<tr>
<td>LNPSG</td>
<td>441.2456</td>
<td>469.2405</td>
<td>NP</td>
<td>184.1081</td>
<td>212.1030</td>
<td>NPS</td>
<td>271.1401</td>
<td>299.1350</td>
</tr>
<tr>
<td>NPSG</td>
<td>328.1615</td>
<td>356.1565</td>
<td>PS</td>
<td>157.0972</td>
<td>185.0921</td>
<td>PSG</td>
<td>214.1189</td>
<td>242.1135</td>
</tr>
<tr>
<td>SG</td>
<td>117.0659</td>
<td>145.0608</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Spot 57. Vacuolar H\(^+\)-ATPase A1 subunit isoform

Peptide View

MS/MS Fragmentation of LHDDLIAGFR
Found in [gi|224682944], KS18003A11 KS18 Capsicum annum cDNA, mRNA sequence
Translated in frame 2 (nucleic acid sequence)

Match to Query 1: 1155.593676 from(1156.600952,1+)
Title: "Ha2_MS2_[1156.615]_0001"
Data file Automatically uploaded data

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 300 to 1150 Da Full range
Label all possible matches Label matches used for scoring

Monoisotopic mass of neutral peptide Mr(calc): 1155.6037
Fixed modifications: Carboxydymethyl (C) (apply to specified residues or termini only)
Ions Score: 58 Expect: 0.013
Matches : 15/114 fragment ions using 21 most intense peaks  

<table>
<thead>
<tr>
<th>#</th>
<th>Immon.</th>
<th>a</th>
<th>a⁰</th>
<th>b</th>
<th>b⁰</th>
<th>Seq.</th>
<th>y</th>
<th>y⁰</th>
<th>y⁰</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>86.0964</td>
<td>86.0964</td>
<td>114.0913</td>
<td>L</td>
<td>1043.5269</td>
<td>1026.5003</td>
<td>1025.5163</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>110.0713</td>
<td>223.1553</td>
<td>251.1503</td>
<td>H</td>
<td>906.4680</td>
<td>889.4414</td>
<td>888.4574</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>88.0393</td>
<td>338.1823</td>
<td>320.1717</td>
<td>366.1772</td>
<td>348.1666</td>
<td>D</td>
<td>791.4410</td>
<td>774.4145</td>
<td>773.4305</td>
</tr>
<tr>
<td>4</td>
<td>88.0393</td>
<td>453.2092</td>
<td>435.1987</td>
<td>481.2041</td>
<td>463.1936</td>
<td>D</td>
<td>676.4141</td>
<td>659.3875</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>86.0964</td>
<td>566.2933</td>
<td>548.2827</td>
<td>594.2882</td>
<td>576.2776</td>
<td>L</td>
<td>563.3300</td>
<td>546.3035</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>86.0964</td>
<td>679.3774</td>
<td>661.3668</td>
<td>707.3723</td>
<td>689.3617</td>
<td>I</td>
<td>450.2459</td>
<td>433.2194</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>44.0495</td>
<td>750.4145</td>
<td>732.4039</td>
<td>778.4094</td>
<td>760.3988</td>
<td>A</td>
<td>379.2088</td>
<td>362.1823</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>30.0338</td>
<td>807.4359</td>
<td>789.4254</td>
<td>835.4308</td>
<td>817.4203</td>
<td>G</td>
<td>322.1874</td>
<td>305.1608</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>120.0808</td>
<td>954.5043</td>
<td>936.4938</td>
<td>982.4993</td>
<td>964.4887</td>
<td>P</td>
<td>175.1190</td>
<td>158.0924</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>129.1135</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Spot 57. Vacuolar H\(^+\)-ATPase A1 subunit isoform (continued)

<table>
<thead>
<tr>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD</td>
<td>225.0982</td>
<td>253.0931</td>
<td>HDD</td>
<td>340.1252</td>
<td>368.1201</td>
<td>HDDL</td>
<td>453.2092</td>
<td>481.2041</td>
</tr>
<tr>
<td>HDDL</td>
<td>566.2933</td>
<td>594.2982</td>
<td>HDDLIA</td>
<td>637.3304</td>
<td>665.3233</td>
<td>HDDLIA</td>
<td>694.3519</td>
<td>722.3468</td>
</tr>
<tr>
<td>DD</td>
<td>203.0662</td>
<td>231.0612</td>
<td>DDL</td>
<td>316.1503</td>
<td>344.1452</td>
<td>DDL</td>
<td>429.2344</td>
<td>457.2293</td>
</tr>
<tr>
<td>DDDL</td>
<td>500.2715</td>
<td>528.2664</td>
<td>DDLIAG</td>
<td>557.2930</td>
<td>585.2879</td>
<td>DL</td>
<td>201.1234</td>
<td>229.1183</td>
</tr>
<tr>
<td>DLI</td>
<td>314.2074</td>
<td>342.2023</td>
<td>DLIA</td>
<td>385.2445</td>
<td>413.2395</td>
<td>DLIAG</td>
<td>442.2660</td>
<td>470.2609</td>
</tr>
<tr>
<td>DLIAGF</td>
<td>589.3344</td>
<td>617.3293</td>
<td>LIA</td>
<td>199.1805</td>
<td>227.1754</td>
<td>LIA</td>
<td>270.2176</td>
<td>298.2125</td>
</tr>
<tr>
<td>LIAG</td>
<td>327.2391</td>
<td>355.2340</td>
<td>LIAGF</td>
<td>474.3075</td>
<td>502.3024</td>
<td>IA</td>
<td>157.1335</td>
<td>185.1285</td>
</tr>
<tr>
<td>IAG</td>
<td>214.1550</td>
<td>242.1499</td>
<td>IAGF</td>
<td>361.2234</td>
<td>389.2183</td>
<td>AG</td>
<td>101.0709</td>
<td>129.0659</td>
</tr>
<tr>
<td>AGF</td>
<td>248.1394</td>
<td>276.1343</td>
<td>GF</td>
<td>177.1022</td>
<td>205.0972</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Graph](image-url)
Spot 9. Adenosine kinase isoform 1S

Peptide View

MS/MS Fragmentation of ALPYMDVFVFNTEAR
Found in gH15186292, 183A01 Mature tuber lama ZAP Solanum tuberosum cDNA, mRNA sequence
Translated in frame 3 (nucleic acid sequence)

Match to Query 1: 1875.122724 from(1876.130000.1+)
Title: 1: Scan 1 (rt=0, well=M9)
Data file tempfile

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from [100] to [1900] Da [Full range]
Label all possible matches [ ] Label matches used for scoring [ ]

Monoisotopic mass of neutral peptide Mr(calc): 1874.8509
Fixed modifications: Carboxamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
MS : Oxidation (M), with neutral losses 63.9983(shown in table), 0.0000
Ions Score: 49 Expect: 0.047
Matches : 22/320 fragment ions using 32 most intense peaks (help)

<table>
<thead>
<tr>
<th>#</th>
<th>Immon.</th>
<th>a</th>
<th>a*</th>
<th>a0</th>
<th>b</th>
<th>b*</th>
<th>b0</th>
<th>Seq.</th>
<th>y</th>
<th>y*</th>
<th>y0</th>
<th>#</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44.0495</td>
<td>44.0495</td>
<td>72.0444</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>86.0964</td>
<td>157.1335</td>
<td>185.1285</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>70.0651</td>
<td>254.1863</td>
<td>282.1812</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>136.0757</td>
<td>417.2496</td>
<td>445.2445</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>56.0495</td>
<td>500.2867</td>
<td>528.2817</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>88.0393</td>
<td>615.3137</td>
<td>597.3031</td>
<td>643.3086</td>
<td>625.2980</td>
<td>D</td>
<td>1284.5855</td>
<td>1267.5596</td>
<td>1266.5749</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>120.0808</td>
<td>762.3821</td>
<td>744.3715</td>
<td>790.3770</td>
<td>772.3665</td>
<td>L</td>
<td>1169.5586</td>
<td>1152.5320</td>
<td>1151.5480</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>72.0808</td>
<td>861.4505</td>
<td>843.4400</td>
<td>889.4454</td>
<td>871.4349</td>
<td>P</td>
<td>1022.4901</td>
<td>1005.4636</td>
<td>1004.4796</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>120.0808</td>
<td>1008.5189</td>
<td>990.5084</td>
<td>1036.5138</td>
<td>1018.5033</td>
<td>F</td>
<td>923.4217</td>
<td>906.3952</td>
<td>905.4112</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>30.0338</td>
<td>1065.5404</td>
<td>1047.5298</td>
<td>1093.5353</td>
<td>1075.5247</td>
<td>V</td>
<td>776.3533</td>
<td>759.3268</td>
<td>758.3428</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>87.0553</td>
<td>1179.5833</td>
<td>1162.5568</td>
<td>1161.5728</td>
<td>1207.5782</td>
<td>1190.5517</td>
<td>1189.5677</td>
<td>N</td>
<td>719.3319</td>
<td>702.3053</td>
<td>701.3213</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>102.0550</td>
<td>1308.6259</td>
<td>1291.5994</td>
<td>1290.6154</td>
<td>1336.6208</td>
<td>1319.5943</td>
<td>1318.6103</td>
<td>E</td>
<td>605.2889</td>
<td>588.2624</td>
<td>587.2784</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>74.0600</td>
<td>1409.6736</td>
<td>1392.6470</td>
<td>1391.6630</td>
<td>1437.6685</td>
<td>1420.6420</td>
<td>1419.6579</td>
<td>T</td>
<td>476.2463</td>
<td>459.2198</td>
<td>458.2358</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>102.0550</td>
<td>1538.7162</td>
<td>1521.6896</td>
<td>1520.7056</td>
<td>1566.7111</td>
<td>1549.6846</td>
<td>1548.7005</td>
<td>E</td>
<td>375.1987</td>
<td>358.1721</td>
<td>357.1881</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>44.0495</td>
<td>1609.7333</td>
<td>1592.7268</td>
<td>1591.7427</td>
<td>1637.7482</td>
<td>1620.7217</td>
<td>1619.7377</td>
<td>A</td>
<td>246.1561</td>
<td>229.1295</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>129.1135</td>
<td>1812.7731</td>
<td>1795.7666</td>
<td>1794.7825</td>
<td>1841.7881</td>
<td>1824.7611</td>
<td>1823.7769</td>
<td>R</td>
<td>175.1190</td>
<td>158.0924</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
Spot 9. Adenosine kinase isoform 1S (continued)

<table>
<thead>
<tr>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP</td>
<td>183.1492</td>
<td>211.1441</td>
<td>LPY</td>
<td>346.2125</td>
<td>374.2074</td>
<td>LPYM</td>
<td>429.2496</td>
<td>457.2445</td>
</tr>
<tr>
<td>LPYMD</td>
<td>544.2766</td>
<td>572.2715</td>
<td>LPYMD</td>
<td>691.3450</td>
<td>719.3399</td>
<td>PY</td>
<td>233.1285</td>
<td>261.1234</td>
</tr>
<tr>
<td>PY</td>
<td>316.1656</td>
<td>344.1605</td>
<td>PYMD</td>
<td>431.1925</td>
<td>459.1874</td>
<td>PYMD</td>
<td>578.2609</td>
<td>606.2558</td>
</tr>
<tr>
<td>PYMDV</td>
<td>677.3293</td>
<td>705.3243</td>
<td>YM</td>
<td>219.1128</td>
<td>247.1077</td>
<td>YMD</td>
<td>334.1397</td>
<td>362.1347</td>
</tr>
<tr>
<td>YMDF</td>
<td>481.2082</td>
<td>509.2031</td>
<td>YMDFV</td>
<td>580.2766</td>
<td>608.2715</td>
<td>MD</td>
<td>171.0764</td>
<td>199.0713</td>
</tr>
<tr>
<td>MDF</td>
<td>318.1448</td>
<td>346.1397</td>
<td>MDFV</td>
<td>417.2132</td>
<td>445.2082</td>
<td>MDFV</td>
<td>564.2817</td>
<td>592.2766</td>
</tr>
<tr>
<td>MDFVFG</td>
<td>621.3031</td>
<td>649.2980</td>
<td>DF</td>
<td>235.1077</td>
<td>263.1026</td>
<td>DFV</td>
<td>334.1761</td>
<td>362.1710</td>
</tr>
<tr>
<td>DFV</td>
<td>481.2445</td>
<td>509.2395</td>
<td>DFVFG</td>
<td>538.2660</td>
<td>566.2609</td>
<td>DFVFG</td>
<td>652.3089</td>
<td>680.3039</td>
</tr>
<tr>
<td>FV</td>
<td>219.1492</td>
<td>247.1441</td>
<td>FV</td>
<td>366.2176</td>
<td>394.2125</td>
<td>FVFG</td>
<td>423.2391</td>
<td>451.2340</td>
</tr>
<tr>
<td>FVFG</td>
<td>537.2820</td>
<td>565.2769</td>
<td>FVFGN</td>
<td>666.3246</td>
<td>694.3195</td>
<td>VF</td>
<td>219.1492</td>
<td>247.1441</td>
</tr>
<tr>
<td>VFG</td>
<td>276.1707</td>
<td>304.1656</td>
<td>VFHN</td>
<td>390.2136</td>
<td>418.2085</td>
<td>VFH</td>
<td>519.2562</td>
<td>547.2511</td>
</tr>
<tr>
<td>VFGNET</td>
<td>620.3039</td>
<td>648.2988</td>
<td>FG</td>
<td>177.1022</td>
<td>205.0972</td>
<td>FGN</td>
<td>291.1452</td>
<td>319.1401</td>
</tr>
<tr>
<td>FGNE</td>
<td>420.1878</td>
<td>448.1827</td>
<td>FGNET</td>
<td>521.2354</td>
<td>549.2304</td>
<td>FGNET</td>
<td>630.2780</td>
<td>678.2729</td>
</tr>
<tr>
<td>GN</td>
<td>144.0768</td>
<td>172.0717</td>
<td>GNE</td>
<td>273.1193</td>
<td>301.1143</td>
<td>GNET</td>
<td>374.1670</td>
<td>402.1619</td>
</tr>
<tr>
<td>GNETE</td>
<td>503.2096</td>
<td>531.2045</td>
<td>GNETEA</td>
<td>574.2467</td>
<td>602.2416</td>
<td>NE</td>
<td>216.0979</td>
<td>244.0928</td>
</tr>
<tr>
<td>NET</td>
<td>317.1456</td>
<td>345.1405</td>
<td>NETE</td>
<td>446.1882</td>
<td>474.1831</td>
<td>NETEA</td>
<td>517.2253</td>
<td>545.2202</td>
</tr>
<tr>
<td>ET</td>
<td>203.1026</td>
<td>231.0975</td>
<td>ETE</td>
<td>332.1452</td>
<td>360.1401</td>
<td>ETEA</td>
<td>403.1823</td>
<td>431.1773</td>
</tr>
<tr>
<td>TE</td>
<td>203.1026</td>
<td>231.0975</td>
<td>TEA</td>
<td>274.1397</td>
<td>302.1347</td>
<td>EA</td>
<td>173.0921</td>
<td>201.0870</td>
</tr>
</tbody>
</table>

[Graphs showing error distributions with mass and RFS error values]
Spot 12. Cell division control protein 48 homolog A

Peptide View

MS/MS Fragmentation of KYQAFQTLQQSR
Found in CD48_ARATH, Cell division control protein 48 homolog A OS=Arabidopsis thaliana GN=CDC48A PE=1 SV=1

Match to Query 1: 1568.053724 from(1569.061000,1+)
Title: 1: Scan 1 (rt=0, well=F5)
Data file tempfile

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 200 to 1600 Da Full range
Label all possible matches Label matches used for scoring

Monoisotopic mass of neutral peptide Mr(calc): 1567.8107
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 61 Expect: 4.6e-09
Matches to Fragment ions using 39 most intense peaks  (help)

<table>
<thead>
<tr>
<th>#</th>
<th>Immon.</th>
<th>a</th>
<th>a*</th>
<th>a0</th>
<th>b</th>
<th>b*</th>
<th>b0</th>
<th>Seq.</th>
<th>y</th>
<th>y*</th>
<th>y0</th>
<th>#</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>101.1073</td>
<td>101.1073</td>
<td>84.0808</td>
<td>129.1022</td>
<td>112.0757</td>
<td>K</td>
<td></td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>136.0757</td>
<td>264.1707</td>
<td>247.1441</td>
<td>292.1656</td>
<td>275.1390</td>
<td>Y</td>
<td>1440.7230</td>
<td>1423.6965</td>
<td>1422.7124</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>101.0709</td>
<td>392.2292</td>
<td>375.2027</td>
<td>420.2241</td>
<td>403.1976</td>
<td>Q</td>
<td>1277.6597</td>
<td>1260.6331</td>
<td>1259.6491</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>44.0495</td>
<td>463.2663</td>
<td>446.2398</td>
<td>491.2613</td>
<td>474.2347</td>
<td>A</td>
<td>1149.6011</td>
<td>1132.5746</td>
<td>1131.5905</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>120.0808</td>
<td>610.3348</td>
<td>593.3082</td>
<td>638.3297</td>
<td>621.3031</td>
<td>F</td>
<td>1078.5640</td>
<td>1061.5374</td>
<td>1060.5534</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>44.0495</td>
<td>681.3719</td>
<td>664.3453</td>
<td>709.3668</td>
<td>692.3402</td>
<td>A</td>
<td>931.4956</td>
<td>914.4690</td>
<td>913.4850</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>101.0709</td>
<td>809.4305</td>
<td>792.4039</td>
<td>837.4254</td>
<td>820.3988</td>
<td>Q</td>
<td>860.4585</td>
<td>843.4319</td>
<td>842.4479</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>74.0600</td>
<td>910.4781</td>
<td>893.4516</td>
<td>892.4676</td>
<td>938.4730</td>
<td>921.4465</td>
<td>920.4625</td>
<td>T</td>
<td>732.3999</td>
<td>715.3733</td>
<td>714.3893</td>
<td>6</td>
</tr>
<tr>
<td>9</td>
<td>86.0964</td>
<td>1023.5622</td>
<td>1006.5356</td>
<td>1005.5516</td>
<td>1051.5571</td>
<td>1034.5306</td>
<td>1033.5465</td>
<td>L</td>
<td>631.3522</td>
<td>614.3257</td>
<td>613.3416</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>101.0709</td>
<td>1151.6208</td>
<td>1134.5942</td>
<td>1133.6102</td>
<td>1179.6157</td>
<td>1162.5891</td>
<td>1161.6051</td>
<td>Q</td>
<td>518.2681</td>
<td>501.2416</td>
<td>500.2576</td>
<td>4</td>
</tr>
<tr>
<td>11</td>
<td>101.0709</td>
<td>1279.6794</td>
<td>1262.6528</td>
<td>1261.6688</td>
<td>1307.6743</td>
<td>1290.6477</td>
<td>1289.6637</td>
<td>Q</td>
<td>390.2096</td>
<td>373.1830</td>
<td>372.1990</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>60.0444</td>
<td>1366.7114</td>
<td>1349.6848</td>
<td>1348.7008</td>
<td>1394.7063</td>
<td>1377.6797</td>
<td>1376.6957</td>
<td>S</td>
<td>262.1510</td>
<td>245.1244</td>
<td>244.1404</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>129.1135</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R</td>
<td>175.1190</td>
<td>158.0924</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>
Spot 12. Cell division control protein 48 homolog A (continued)

<table>
<thead>
<tr>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
<th>Seq</th>
<th>ya</th>
<th>yb</th>
</tr>
</thead>
<tbody>
<tr>
<td>YQ</td>
<td>264.1343</td>
<td>292.1292</td>
<td>YQA</td>
<td>335.1714</td>
<td>363.1663</td>
<td>YQAF</td>
<td>482.2398</td>
<td>510.2347</td>
</tr>
<tr>
<td>YQAFA</td>
<td>553.2769</td>
<td>581.2718</td>
<td>YQAFAQ</td>
<td>681.3355</td>
<td>709.3304</td>
<td>QA</td>
<td>172.1081</td>
<td>200.1030</td>
</tr>
<tr>
<td>QAF</td>
<td>319.1765</td>
<td>347.1714</td>
<td>QAFA</td>
<td>390.2136</td>
<td>418.2085</td>
<td>QAFAQ</td>
<td>518.2722</td>
<td>546.2671</td>
</tr>
<tr>
<td>QAFAQT</td>
<td>619.3198</td>
<td>647.3148</td>
<td>AF</td>
<td>191.1179</td>
<td>219.1128</td>
<td>AFA</td>
<td>262.1550</td>
<td>290.1499</td>
</tr>
<tr>
<td>AFAQ</td>
<td>390.2136</td>
<td>418.2085</td>
<td>AFAQT</td>
<td>491.2613</td>
<td>519.2562</td>
<td>AFAQTL</td>
<td>604.3453</td>
<td>632.3402</td>
</tr>
<tr>
<td>FA</td>
<td>191.1179</td>
<td>219.1128</td>
<td>FAQ</td>
<td>319.1765</td>
<td>347.1714</td>
<td>FAQT</td>
<td>420.2241</td>
<td>448.2191</td>
</tr>
<tr>
<td>FAQTL</td>
<td>533.3082</td>
<td>561.3031</td>
<td>FAQTLQ</td>
<td>661.3668</td>
<td>689.3617</td>
<td>AQ</td>
<td>172.1081</td>
<td>200.1030</td>
</tr>
<tr>
<td>QTLQ</td>
<td>443.2613</td>
<td>471.2562</td>
<td>QTLQQ</td>
<td>571.3198</td>
<td>599.3148</td>
<td>QTLQQS</td>
<td>658.3519</td>
<td>686.3468</td>
</tr>
<tr>
<td>TLQQS</td>
<td>530.2933</td>
<td>558.2882</td>
<td>LQ</td>
<td>214.1550</td>
<td>242.1499</td>
<td>LQQ</td>
<td>342.2136</td>
<td>370.2085</td>
</tr>
<tr>
<td>LQQS</td>
<td>429.2456</td>
<td>457.2405</td>
<td>QQ</td>
<td>229.1295</td>
<td>257.1244</td>
<td>QQS</td>
<td>316.1615</td>
<td>344.1565</td>
</tr>
<tr>
<td>QS</td>
<td>188.1030</td>
<td>216.0979</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Error Graph]

RMS error 134 ppm
Supplementary Data 2. Results of MS/MS ion search and BLAST search for protein identification.

<table>
<thead>
<tr>
<th>Spot No</th>
<th>Protein ID</th>
<th>EST (Accession No.)*</th>
<th>Homolog (Accession No.)</th>
<th>Species</th>
<th>m/z</th>
<th>Sequence</th>
<th>Delta Miss</th>
<th>Score</th>
<th>Expect</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Isocitrate dehydrogenase [NADP]</td>
<td>ND</td>
<td>IDHC_SOLTU</td>
<td>Solanum tuberosum</td>
<td>1797.89</td>
<td>GGETSNIASIFAWTR</td>
<td>0.02</td>
<td>0</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Pyrophosphate-fructose 6-phosphate 1-phosphotransferase subunit beta</td>
<td>ND</td>
<td>PFPB_SOLTU</td>
<td>Solanum tuberosum</td>
<td>1660.17</td>
<td>YVVLTPEFIPYPR</td>
<td>0.29</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>74</td>
<td>Pyrophosphate-fructose 6-phosphate 1-phosphotransferase subunit beta</td>
<td>ND</td>
<td>PFPB_SOLTU</td>
<td>Solanum tuberosum</td>
<td>1660.03</td>
<td>YVVLTPEFIPYPR</td>
<td>0.15</td>
<td>0</td>
<td>39</td>
</tr>
<tr>
<td>63</td>
<td>Enolase</td>
<td>ND</td>
<td>ENO_SOLL</td>
<td>Solanum lycopersicum</td>
<td>1660.17</td>
<td>YVVLTPEFIPYPR</td>
<td>0.15</td>
<td>0</td>
<td>28</td>
</tr>
<tr>
<td>67</td>
<td>Fructose-bisphosphate aldolase-like protein</td>
<td>gi</td>
<td>2504723</td>
<td>XP_004246552</td>
<td>Solanum lycopersicum</td>
<td>2052.28</td>
<td>IGANEPSQLAINENANGLAR</td>
<td>0.23</td>
<td>0</td>
</tr>
<tr>
<td>17</td>
<td>Dihydroxyacetone phosphate dehydrogenase precursor</td>
<td>gi</td>
<td>5894612</td>
<td>NP_001275339</td>
<td>Solanum tuberosum</td>
<td>2064.36</td>
<td>FLSPSEISVDTVEGGNSVVK</td>
<td>0.31</td>
<td>1</td>
</tr>
</tbody>
</table>

**CARBOHYDRATE METABOLISM**

**AMINO ACID/PROTEIN METABOLISM**

**DEFENSE RESPONSE**

**ETC/ATP INVOLVED REACTION**

**Others**

---

*Spot number that corresponds to Prodigy rank number.

*Accession number of the top hit sequence from the Solanaceae EST database. ND: Not determined, as a homolog was directly hit from SwissProt.

*Accession number of the top hit homolog from MS/MS ion search (MIS) using SwissProt database or MIS followed by EST-based BLAST search using non-redundant protein.

Source organism of the 'homolog' indicated in the left column.

Oxidized Met is underlined.

Number of missed cleavage sites in the tryptic fragment.