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University of Nevada, Reno

Protein Expression Analysis in the Resurrection Plant, *Selaginella lepidophylla*

A thesis submitted in partial fulfillment
Of the requirements for the degree of

Bachelor of Science in Biochemistry and Molecular Biology

By
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THE HONORS PROGRAM

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Abstract

Understanding the mechanisms used by plants to survive stressful environmental conditions, such as water deficits due to drought is vital to the development of genetic engineering strategies to improve or retain agricultural productivity in the face of increasing environmental insults, changes in rainfall patterns and global warming. Desiccation tolerance is a rare adaptive response of resurrection plants to exist in environments with intermittent drought by entering into a state of metabolic inactivity, wherein vegetative structures are preserved under air-dried conditions. Upon rewatering, these plants resume their metabolic activities and repair any damage that occurred while in the dry state. The mechanistic basis of desiccation tolerance can be better understood by studying it using integrative functional genomics approaches including transcriptomics, proteomics, and metabolomics. In this study, protein expression patterns within fully hydrated and desiccated tissues of *Selaginella lepidophylla* were investigated using a gel-based proteomics approach. A phenol-based protein extraction protocol was optimized for *S. lepidophylla* tissues by including a series of washing steps with methanol, acetone and ether in order to remove membrane lipids and polyphenolics. Protein expression profiles were then compared using two-dimensional difference polyacrylamide gel electrophoresis (2D-DIGE). The 2D-DIGE analysis revealed that 130 proteins that were differentially expressed with 107 proteins showing increased abundance and 23 showing decreased abundance in the dry state compared with the hydrated state. Late embryogenesis abundant and heat shock proteins and reactive oxygen scavenging enzymes were overrepresented in the dried state.

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1 Literature Review

Desiccation tolerance is an adaptive response that the first land plants acquired to be able to survive drying. This trait could be present in vegetative or reproductive structures of the plant or both. A desiccation tolerant plant that achieves direct equilibrium of cell water content with that of the environment (less than 5% air relative humidity) is called poikilohydry (Bernacchia and Furini, 2004). In such plants in the dry state, the membranes within cell structures remain intact, but during the hydration process, cells suffer alterations that can damage them. As a consequence, the plant needs specific adaptations to survive those alterations (Oliver et al., 2005). The molecular genetic and biochemical basis of these adaptations are not fully understood.

Selaginella lepidophylla is desiccation tolerant tracheophyte native from xeric areas and low elevation communities in central and north America (Auken and Bush, 1992). This plant is considered a resurrection plant because during drought periods it loses 95% of its water content and stops its metabolic activities until water conditions are favorable for photosynthetic processes to resume (Iturriaga et al., 2006). This species is able to survive for two to three years under dry conditions (Eickmeier, 1979). There have been several physiological studies done on *S. lepidophylla* and other resurrection plants. Among those studies, Bergtrom et al. (1982) determined that during the hydration process, *S. lepidophylla* cells undergo drastic changes. During the first six hours of hydration, polyphenolics condense in cytoplasmic vacuoles, respiration resumes, and the levels of photosynthesis and CO_2 fixation are low. During the next 8-to-12 hours of hydration,

polysomes form in the cytoplasm, and chloroplasts and a central vacuole appear, and the activity of ribulose-1,5-bisphosphate carboxylase oxygenase (RUBISCO) increases. During the next 12-to-24 hours, the grana forms, photosynthetic rates and RUBISCO activity increases. In response to the accumulation of polyphenolics, studies done on the woody resurrection plant, *Myrothamnus flabellifolia*, have shown that certain compounds, such as polyphenolics, might play an important role in protection of artificial membranes against desiccation and free-radical-induced oxidation (Moore et al., 2007). This protection system in *M. flabellifolia* could be related to the progressive accumulation of zeaxanthin and redox shifts of the antioxidants glutathione and ascorbate to their oxidized forms, the concentration of violaxanthin, β-carotene, ascorbate, α-tocopherol and the decrease in the activity of glutathione reductase. All these changes in the concentration and activity of plant antioxidants translate into the break-down of antioxidant defense mechanism (Kranner et al., 2002).

The accumulation of certain metabolites in resurrection plants has been studied at diverse levels. In the resurrection plant *Xerophyta viscose* sucrose accumulates upon water deficit. In addition, differences in the carbohydrate profiles of individual plants have been reported, which are likely associated with differences in the innate competencies among individuals within wild populations. The innate differences might be caused by different environmental conditions under which individual resurrection plants were dried and differences in age. The accumulation of sucrose and raffinose family oligosaccharides (RFOs) during the dry state is thought to contribute to the stabilization of proteins and membranes during desiccation-induced vitrification in the cytoplasm (Peters et al., 2007;

Wingler, 2002). The accumulation of sugars and late embryogenesis (LEA) proteins in resurrection plants in the dry state has been documented for other species of tracheophyte resurrection plants. The sugar accumulation has been associated with protection in the dry states and LEA accumulation with repair of desiccation-induced damage (Bernacchia and Furini, 2004; Jiang et al., 2007; Oliver et al., 2005; Scott, 2000; Wang et al., 2009b). *S. lepidophylla* accumulates specific molecules before dehydration that are presumed to afford cellular protection and proteins that allow recovery of the cells when rehydration occurs (Bergtrom et al., 1982; Iturriaga et al., 2006; Jiang et al., 2007). The synthesis of trehalose is catalyzed in two enzymatic steps by the oligomeric subunits trehalose-6-phosphate synthase (TPS). The gene encoding the enzyme TPS (SITPS1) was isolated from cDNA of *S. lepidophylla*, and is thought it could play a major role in stress tolerance in this plant (Zentella et al., 1999).

Because the accumulation of particular metabolites can be associated with specific enzymes, several researchers have studied the enzymatic activities in the dry and hydrated states of resurrection plants in order to understand the resurrection trait. Infrared CO_2 gas analysis and RUBISCO activity measurements in *S. lepidophylla* have shown that full photosynthetic recovery requires 23-to-26 h at temperatures between 15 and 35°C. Dry plants showed substantial RUBISCO activity (60% of the normal activity), which increased after hydration (Eickmeier, 1979). A study conducted by Harten and Eickemeier (1986) showed that the activities of aconitase, citrate synthase, enolase, pyruvate kinase, Ribose-5-P isomerase, RUBISCO, and triose-P dehydrogenase increase in the hydration process in fronds of *S. lepidophylla* (Harten and Eickmeier, 1986).

In addition to enzymatic studies during the hydration process on resurrection plants, there are several molecular genetic studies. Alamillo et al. (1994) showed that cDNA clones isolated from dehydrated vegetative tissues of the resurrection plant *Craterostigma plantagineum* encoded polypeptides with homologies to proteins expressed during late embryogenesis in higher plant embryos. Abscisic acid treatment of leaves and undifferentiated callus tissue also promoted the accumulated of these same cDNA clones. They determined that all the dehydration-induced LEA proteins were located in the cytoplasm except for three groups of them including dsp 21 (CD8 (carrot), group 3 LEA (wheat) and ABA-inducible gene (barley)), dsp 22 (Elips (pea and barley), cbr (*Dunaliella*)) and dsp 34 (*Arabidopsis*), which were localized in the chloroplast and concluded that the pathways leading to water-stress tolerance in embryos and vegetative tissues were similar.

Other studies done on *C. plantagineum* have shown that homeobox leucine zipper genes 3/4/5/6/7 (CPHB) are modulated in expression as a response to dehydration in leaves and roots, which supports the idea that homeodomain leucine zipper genes regulate gene expression leading to desiccation tolerance (Deng et al., 2002). Other group of proteins that show high relevance for plant resistance and acclimation to dehydration and high temperatures is the heat shock proteins (HSPs). The role of HSPs is to prevent the aggregation and unfolding of proteins when the organism is under stress (Ingle et al., 2007; Timperio et al., 2008; Wang et al., 2004). HSPs are involved in other metabolic pathways such as signaling, translation, host-defense mechanisms, carbohydrate and

amino acid metabolism (Ingle et al., 2007; Timperio et al., 2008) Thus, complex responses of resurrection plants to the dehydration process include changes in the gene expression of signaling pathway components, such as transcription factors, HSPs, chaperones and LEA proteins, ROS scavenging enzymes and metabolites, and the synthesis of osmoprotectants, ion and water transporters, and a range of similar process are common in several plant species (Langridge et al., 2006). These complex responses are also likely to be present in *S. lepidophylla* (Iturriaga et al., 2006).

In addition to the enzymatic and molecular genetic studies in resurrection plants, organelle and cytoplasm directed protein synthesis has been shown to be vital for the recovery of photosynthetic processes during rehydration of *S. lepidophylla* (Eickmeier, 1982; Eickmeier, 1988). Studies investigating the impact of different desiccation rates on protein synthesis rates in *S. lepidophylla* showed that there is no effect on the rate at which protein are synthesized during the last hours of the 24 h rehydration process, however, the types of the proteins synthesized during that period differ.

Resurrection plants have been studied at the structural, physiological and molecular genetic levels (Jiang et al., 2007; Moore et al., 2007). Proteomic analysis is one excellent approach to study desiccation tolerance mechanisms because it documents the actual protein content responsible for performing enzymatic, regulatory, and structural functions (Jiang et al., 2007; Qureshi et al., 2007). Jiang et al. (2007) conducted proteomic studies in *Boea hygrometric,* a desiccation tolerant species, and concluded that desiccation tolerant mechanisms are associated with an increased and accumulation of antioxidant,

sugars, and proteins in a specific spatial and temporal manner. Wang et al. (2009b) also completed a limited proteomic study in *Physomitrella patens* under water deficit conditions that showed that cytoskeletal proteins undergo degradation likely resulting in cytoskeletal disassembly and consequent changes in the cell structure. LEA proteins and reactive oxygen species-scavenging enzymes also accumulate indicating that these might help to diminish the damage brought about by desiccation. Proteomic studies done in *Selaginella bryopteris* showed that proteins involved in transport, targeting and degradation increased in relative abundance in dry fronds (Deeba et al., 2009). A comparative proteomic analysis performed by Ingle et al. (2007) confirmed differences in protein content between the poikilochlorophylous, *Xerophyta visoca* and the homoiochlorophyllous *C. plantagineum* showing that *C. plantagineum* does not dismantle its thylakoid membranes or degrade PSII proteins upon dehydration. In that proteomic analysis was suggested that for *X. viscosa* there were three groups of proteins representing different time points in the dehydration process: early-dehydration, late-dehydration and fully-dehydration.

To date, there are no recent reports on protein expression studies in *S. lepidophylla*. I hypothesize that there are differences in protein expression at different stages of dehydration and rehydration in *S. lepidophylla,* which would provide novel insights into the mechanistic basis of the resurrection trait. The analysis of the desiccation tolerance mechanisms is important because farmers in both developed and developing nations are challenged to feed ever-larger populations in the face of increasing environmental stresses like drought. Crop plants generally lack of desiccation tolerance mechanisms. If

the resurrection trait could be introduced into crop species, then catastrophic crop losses in rain-fed agricultural production systems might be prevented or reduced in magnitude. Understanding how this survival mechanism is expressed is key to advance its application to genetically engineered crops.

In this study, I will compare the protein expression patterns between fully hydrated and desiccated tissues of *S. lepidophylla.* Proteins of leaf (frond) and root tissues will be analyzed by two-dimensional difference polyacrylamide gel electrophoresis (2D-DIGE). The results from the DIGE analysis will allow for the detection and quantification of protein expression differences (Alban et al., 2003) at specific stages of the dehydration and rehydration process. Proteins that exhibit significant differences in their relative abundance between the two states and significant difference to the gel background will be identified and then identified by tandem matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS/MS). This project will complement the research done by Iturriaga et al. (2006) on the expressed sequence tags (ESTs) of *S. lepidophylla*, and ongoing mRNA expression and metabolite profiling studies being performed in the Cushman lab. These protein expression profiling studies will improve our understanding of desiccation tolerance mechanisms, because it will provide a snapshot of biochemical processes at a given point in time. This kind of differential analysis will also include the analysis of posttranslational changes in proteins such as phosphorylation (Alban et al. 2003) that mRNA abundance analyses cannot show.

2 Materials and Methods

2.1 Protein extraction

The protein extraction was performed on four biological replicates of *S. lepidophylla* from hydrated and dry states as described by Vincent et al. (2006) with modification. First, frozen whole plants were individually ground under liquid nitrogen using a grinding mill (Retsch MM301, NV, Reno) for 3 to 5 min. After that 10 ml of modified phenol extraction buffer (0.7 M sucrose, 0.5 M Tris-HCl pH 7.5, 50 mM EDTA, 0.1 M KCl, 2% 2-mercaptoethanol and protease inhibitor cocktail, 1% PVPP) was added to each sample (Damerval et al., 1986; Yao et al., 2006). The samples were incubated at 4°C for 10 min, then 10 ml Tris-saturated Phenol pH 7.9 was added, vortexed for 30 sec, and then again incubated for 30 min at 4°C. After that, the samples were centrifuged at 3,650 *x g* at -4°C for 30 min and the phenol (upper) phase was removed. This process was repeated a second time with a volume of extraction buffer equal to the recovered volume of phenol for each sample. Following the second phenol extraction, the protein samples were precipitated from the phenol phase using five volumes 0.1 M methanolic ammonium acetate (0.1 M ammonium acetate in methanol) The samples were left at -20°C overnight followed by a centrifugation at 3,650 *x g* at 4°C for 30 min. The supernatant was then discarded and 5 ml of ice-cold methanol was added to the pellet and the sample vortexed until the pellets were resuspended in the methanol. The samples were then let stand 1 h at -20°C followed by a centrifugation at 3,650 *x g* at 4°C for 30 min, followed by discarding of the supernatant and resuspension of the individual pellets in 5 ml of ice-cold acetone.

This acetone wash was repeated twice for a total of three washes, followed by precipitation of each pellet with 5 ml of ether per sample. This step was repeated twice. After that, the supernatant was discarded and the tubes were left open on the fume hood until all the ether evaporated, then the samples were stored at -20°C until further use.

2.2 Protein quantification

The protein pellets were resuspended using 100 µl of resuspension buffer (7 M urea, 2 M thiourea, 4% 3- *((3-Cholamidopropyl)dimethylammonio))*-1-Propanesulfonic Acid (CHAPS). The samples were diluted 1:10 and 1:20 and assayed using the EZQ protein quantification kit (Bio-Rad) with bovine serum albumin as the standard.

2.3 DIGE sample preparation

DIGE buffer (7 M urea, 2 M thiourea, 4% CHAPS) was added to the samples to a final volume of 98 µl each, then, 1.2 µl 1.5 M Tris-HCl (pH = 9.44) were added. 7.25 µg from each sample were pooled and to that mixture was added 4.8 µl 1.5 M Tris-HCl (pH = 9.44). After that, the pH of each individual sample and the pooled standard was checked using pH 7-14 indicator strips, and 1-2 µl of 0.2-1.0 M HCl was added to them to achieve a pH of ~8.5. Each sample had a final concentration of 58 µg. To each individual sample 1.15 µl of CyDyes^{TM} were added and 4.6 µl of dye was added to the pooled standard as follows: 1A, Cy5; 1B, Cy3; 1C, Cy5; 1D, Cy3; 2A, Cy5; 2B, Cy3; 2C, Cy5; 2D, Cy3; and pooled standard Cy2 (Table 1). Each tube contained approximately 58 µg total protein and 230 pmoles CyDyes^{TM}. The tubes were centrifuged briefly at 3,650 *x g* at 4°C and incubated for 30 min on ice in the dark. At the end of the 30 min incubation, 2 µl of

10 mM Lysine was added to each tube. The tubes were vortexed and put back on ice and in the dark for 10 minutes. The samples were loaded onto the gel in the following order: gel 1 (1B and 2A); gel 2 (1D and 2C); gel 3 (2B and 1C); gel 4 (2D and 1A). To each gel the following was added: ~58 µg first sample, ~58 µg second sample, ~58 µg combined sample, 230 pmoles of each Cy Dye, 7 M Urea, 2M Thiourea, 4% CHAPS, 11.5 mM Tris-HCl (pH = 8.7), 0.001% Bromophenol Blue, 0.2% pH 3-10 ampholytes (Bio-Rad, Hercules; CA), and 50 mM DTT.

Table 1. Labels of the samples with the Cy Dyes. Samples with number code 1 were hydrated material and with code 2 were dry material.

Gel #	Cy Dye 3 labeled sample	Cy Dye 5 labeled sample
1	1B	2A
2	1D	2C
3	2B	1C
4	2D	1A

2.4 Isoelectric focusing

The individual sample mixtures were centrifuged at 16,000 *x g* , 22°C, for 10 minutes. 450 µl of each supernatant was applied to a 24 cm 4-7 IPG strip (Bio-Rad, Hercules; CA). The strip was rehydrated passively overnight (for about 19 ½ hours). Isoelectric focusing was begun with the following program: step 1: 200 V, linear 1 hour; step 2: 500 V, linear, 1 hour; step 3: 1000 V, linear, 1 hour; step 4: 3000 V, rapid, 1 hour; step 5: 10,000 V, linear, 4 hours; step 5: 10,000 V, rapid, 39,000 Vhours and step 6: 500 V, rapid, 24 hours.

2.5 SDS gel electrophoresis

After the IEF, the strips were equilibrated for electrophoresis and placed on gradient gel 8-16% 26 cm x 20 cm from Sigma-Aldrich (St. Louis, Missouri). Electrophoresis was performed under the following conditions: step 1: 40 V constant, 2 hours; step 2: 100 V constant, 15 hrs, 30 min, the conditions were: 100 V, 93 mA and 9 W, when the bromophenol blue front was about ¾ of the way down the gels the program was changed to: 200 V constant 500mA, 250W, these conditions were continued for about 3 /12 hours. The gels were scanned using Typhoon TRIO variable mode imager from GE Healthcare (Uppsala, Sweden) for Cy3 excitation 532 nm, emission 580 nm; Cy5 excitation 633 nm, emission 670 nm and Cy2 excitation 488 nm and emission 520 nm.

2.6 Gel fixation

All gels were fixed in 7% v/v acetic acid, 10% v/v methanol (Destain) overnight. The Destain was removed and ~130 ml of Sypro Ruby Dye (Bio-Rad, Hercules; CA) in ~600 ml Destain was added to the gels. The gels were stained over 2 days and then washed once with Destain solution for several hours and then with water for two hours before imaging on the Typhoon using the Blue (488 nm) laser. After that, the gels were sealed and stored in the refrigerator.

2.7 Comparative 2D-DIGE Image analysis

The images from the DyCydes^{TM} were analyzed using the DyCyder^{TM} software 7.0 from GE Healthcare (Piscataway, NJ). The images were matched to the master image from gel 4, which have the highest amount of spots. The matching was optimized by using the

vector and grind tools of the DyCyder^{TM} software, after that the images were compared by a t-test and ANOVA. Because of the parameters of comparison in this experiment the t-test and ANOVA values were the same.

2.8 Protein Identification by sequencing with tandem MS/MS

Spots that showed an ANOVA value <0.05 and ratios >1.5 and <-1.5 and were not artifacts, were picked for protein identification using MALDI MS/MS. In addition, spots that showed difference from the background in the gel were picked for protein identification. Spots were digested using Investigator^{TM} Proprep^{TM} (Genomic Solutions, Ann Arbor; MI), using a previously described protocol (Rosenfeld et al., 1992) with some modifications. Samples were washed twice with 25mM ammonium Bicarbonate (ABC) and 100% acetonitrile, reduced and alkylated using 10mM DTT and 100mM Iodoacetamide and incubated with 75ng Trypsin in 25mM ABC for 6 hrs at 37 C.

Samples were prepared and spotted onto a MALDI (Matrix Assisted laser Desorption Ionization) target with ZipTipu-C18 (Millipore, Billerica; MA). Samples were aspirated and dispensed 3 times and eluted with 70% ACN, 0.2% formic acid and overlaid with 0.5µl 5mg/ml MALDI matrix (α-Cyano-4-hydroxycinnamic acid) and 10mM ammonium phosphate. All mass spectrometric data was collected using an ABI 4700 MALDI TOF/TOF (Applied Biosystems, Foster City; CA). The data was acquired in reflector mode from a mass range of 700 – 4000 Daltons and 1250 laser shots were averaged for each mass spectrum. Each sample was internally calibrated on trypsin's autolysis peaks. The twenty most intense ions from the MS analysis, which were not on the exclusion list,

were subjected to MS/MS. For MS/MS analysis the mass range was 70 to precursor ion with a precursor window of -1 to 3 Daltons with an average 5000 laser shots for each spectrum. The data were stored in an Oracle database.

The data was extracted from the Oracle database and a peak list was created by GPS Explorer software (Applied Biosystems, Foster City; CA) from the raw data generated from the ABI 4700. This peak list was based on signal to noise filtering and an exclusion list and included de-isotoping. The resulting file was then searched by Mascot (Matrix Science, Boston; MA). A tolerance of 20ppm was used if the sample was internally calibrated and 200 ppm tolerance if the default calibration was applied. Database search parameters include 1 missed cleavage, oxidation of methionines and carbamidomethylation of cysteines. The same search parameters applied to the Mascot search were used for the EST library from *S. lepidophylla*.

3 Results

3.1 Comparative 2D-DIGE Image analysis and protein identification

Four biological replicates of *S. lepidophylla* plants from the dry and hydrated states were used to perform the 2D-DIGE analyses for a total of eight samples. One of the biological replicates was discarded due to contamination in the hydrated state with bacterial proteins (*Salmonella).* To compare the differentially expressed proteins between dry and hydrated states the 2-DIGE gels were compared as shown in Figures 1, 2 and 3. After the statistical analysis was performed, 1,700 spots were detected in the master gel of which 708 spots displayed differential abundance between dry and hydrated conditions after ANOVA ($p<0.05$). From the 708 differentially expressed spots, 395 were expressed in higher abundance (Average ratio>1.5) in the dry state and 234 were expressed in lower abundance in the dry state (Average ratio<-1.5).

From the spots that were statistically differentially expressed, 175 of the most significantly differentially expressed were picked and then analyzed by MALDI MS/MS (Table 2). In addition, other spots that were not statistically differentially expressed were picked for a total of 274 spots sent to sequencing analysis. Of the 274 spots, 184 had a MOWSE value >80 of which 130 had an ANOVA value smaller than 0.05 (Table 2). Of those 130 spots ($p<0.05$), 109 had a higher abundance in dry state with an average ratio of 1.5 or higher (Table 3), and 3 with an average ratio between 1.3 and 1.5 (Table 4). Of the 130 spots ($p<0.05$), 15 had lower abundance in dry state with an average ratio of 1.5 or higher (Table 5), and 8 with an average ratio between -1.2 and -1.5 (Table 6).

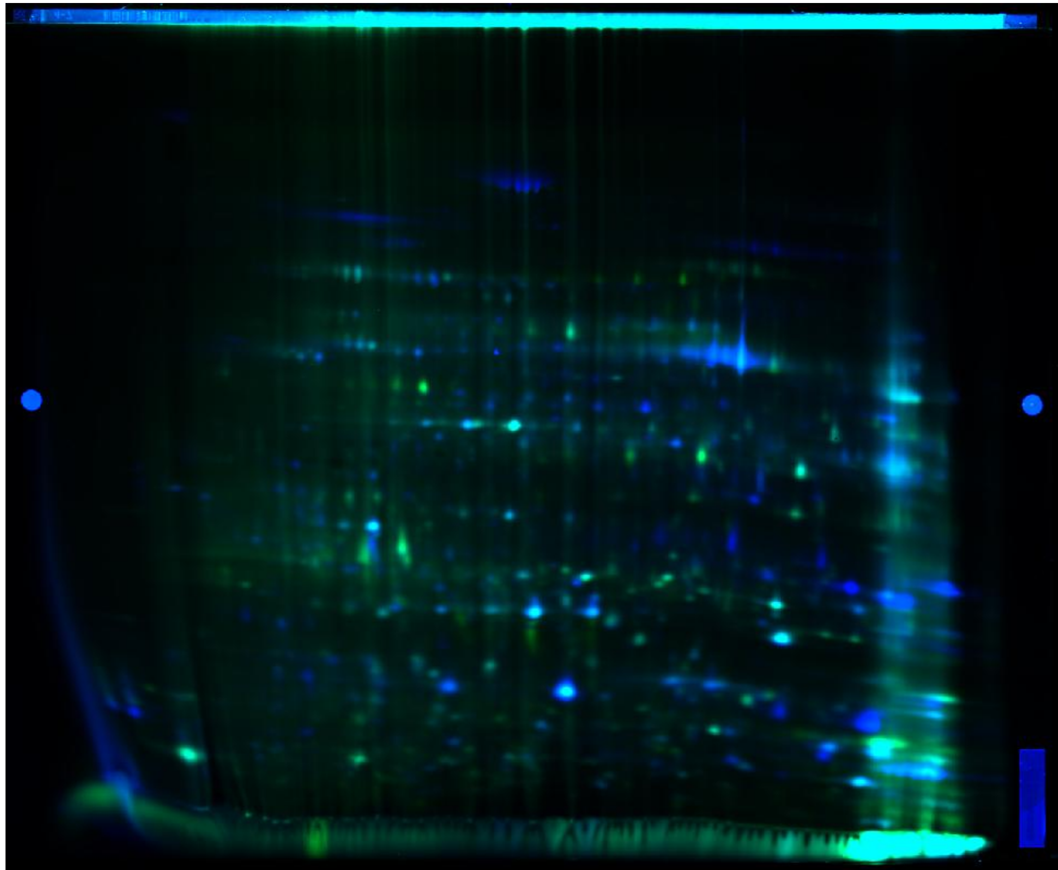

Figure 1. Representative overlay DIGE image of *S. lepidophylla* hydrated (100% RWC) and dry tissue (5% RWC) stained with fluorescent Cy dyes. Blue corresponds to the dry state (Cy3 Labeled) and green to hydrated state (Cy5 Labeled).

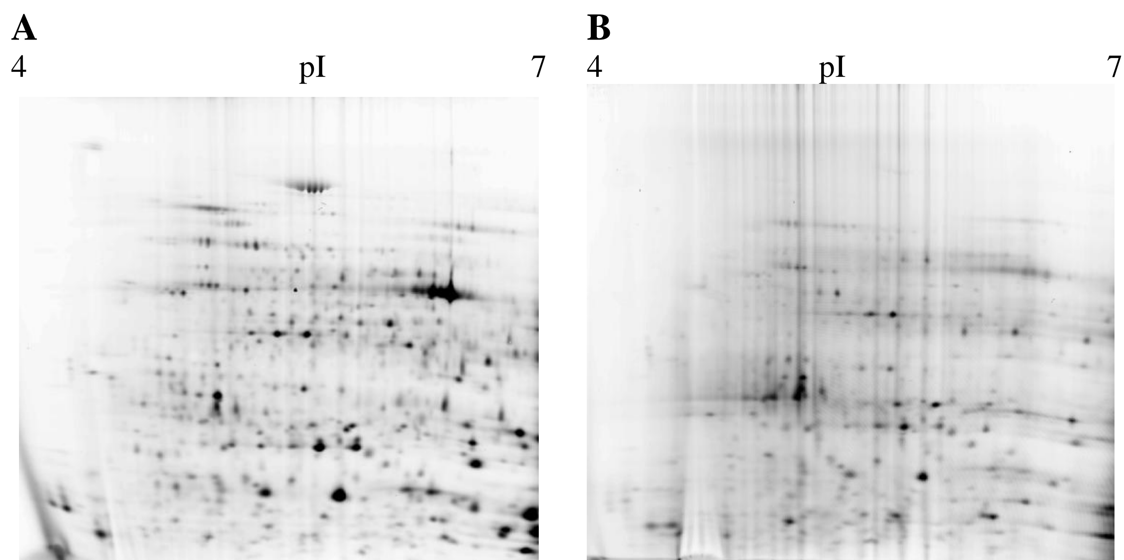

Figure 2. 2-D DIGE image of one of representative biological replicates of *S. lepidophylla* from A) dry state and B) hydrated samples.

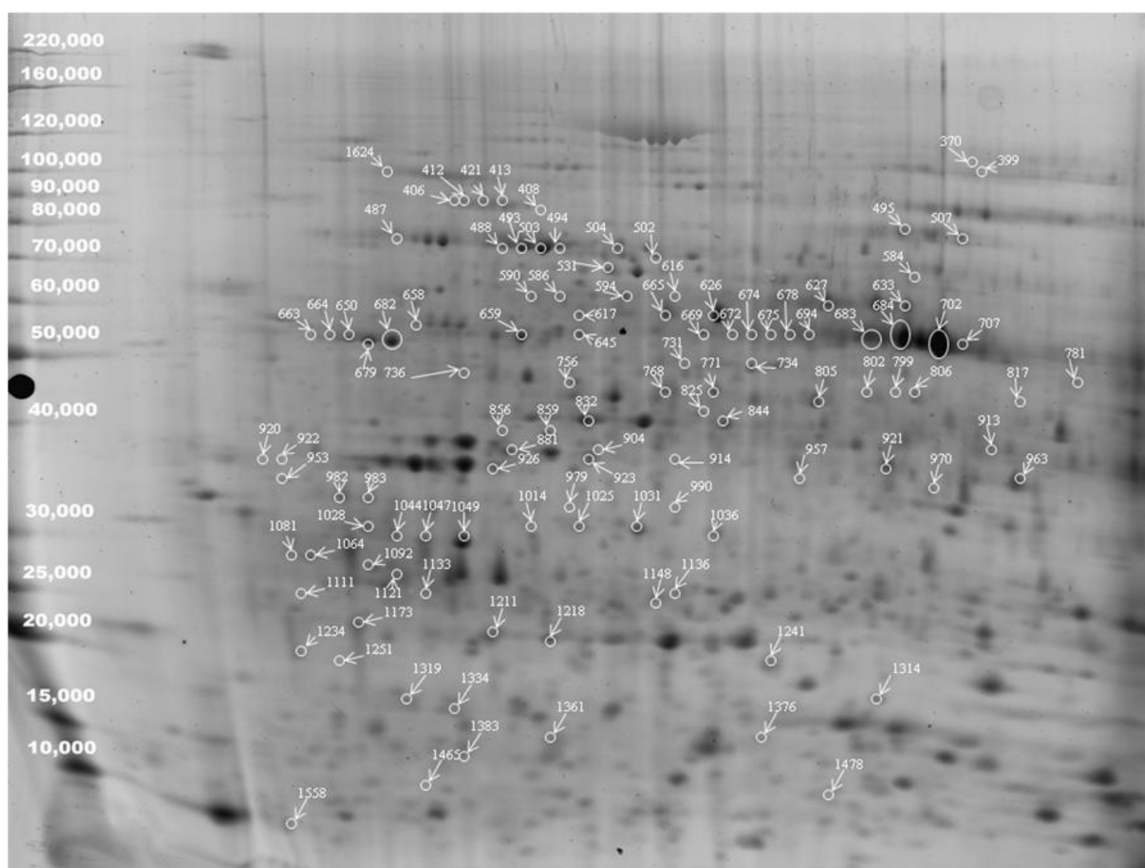

Figure 3. Spots picked subjected to MALDI MS/MS analysis whose abundance differed significantly (p<0.05) between the dry and hydrated states.

Table 2. Summary of the spots detected by 2D-DIGE images, proteins that were sequenced and presented difference in abundance between the dry and hydrated state. *Proteins with p-values<0.05 and **MOWSE score>80 comparing dry and hydrated states.

	Total proteins
Spots on DIGE gel	1,700
Spots with p-val<0.05	708
Spots sequenced	274
Proteins with good IDs**	130
Proteins with high abundance in dry	107
Proteins with low abundance in dry	23

Table 3. Functional categorization of proteins with higher abundance in the dry state compared with the hydrated state that were statistically different and having average ratios greater than 1.5.

SPP	Av. Ratio	Pval	Th Mr (Kd)	Exp Mr (Kd)	Th p *I*	Exp p *I*	Pep	MOWSE Score	C.I.%	Name	Uniprot
Energy											
1135	8.62	0.0128	37.4	30.28	8.37	6.07	10	162	100	Carbonic anhydrase 2	P42737
1078	7.27	0.0164	24.77	33.28	6.58	5.64	2	87	99.95	ribulose-1,5-bisphosphate carboxylase/oxygenase large chain	O03042
1095	7.15	0.012	37.4	32.82	8.37	5.83	11	210	100	Carbonic anhydrase 2	P42737
702	6.67	0.0151	48.52	54.16	6.04	5.86	3	143	100	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	A5X5U0
684	4.4	0.0187	24.77	54.69	6.58	5.78	3	94	99.99	ribulose-1,5-bisphosphate carboxylase/oxygenase large chain	O03042
682	3.93	0.0281	44.85	54.49	8.66	4.72	3	89	99.97	ATP synthase beta chain, mitochondrial, putative	Q9C5A9
707	3.3	0.0175	24.77	53.74	6.58	5.90	3	85	99.92	ribulose-1,5-bisphosphate carboxylase/oxygenase large chain	O03042
683	2.71	0.03	24.77	54.85	6.58	5.73	3	90	99.98	ribulose-1,5-bisphosphate carboxylase/oxygenase large chain	O03042
659	2.7	0.0147	44.85	54.85	8.66	5.00	18	693	100	ATP synthase beta chain, mitochondrial, putative /	Q9C5A9
645	2.47	0.012	37.93	55.29	5.16	5.10	15	362	100	V-type proton ATPase subunit B3, vacuolar	Q8W4E2
617	2.07	0.0225	47.45	57.93	6.18	5.10	13	112	99.99	ribulose-1,5-bisphosphate carboxylase/oxygenase	Q8HT76
756	2.03	0.0266	34.31	50.24	6.32	5.10	14	187	100	RUBISCO activase (RCA), putative	Q3EBJ5
1133	1.98	7.58E-03	32.87	30.95	7.9	4.84	10	456	100	Putative chlorophyll a/b binding protein	Q9SHR7
664	1.8	0.046	35.06	55.06	5.53	4.62	8	85	96.79	ATP synthase subunit beta	A8Y662

694	1.76	0.0381	24.77	54.83	6.58	5.65	3	109	100	ribulose-1,5-bisphosphate carboxylase/oxygenase large chain	O03042
832	1.74	0.0403	59.34	46.56	9.19	5.13	21	758	100	RUBISCO activase (RCA), putative	Q3EBJ5
672	1.68	0.0305	48.06	55.7	6.42	5.46	18	286	100	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	Q8MGI7
663	1.59	0.0157	49.23	55	5	4.60	9	121	100	ATPase beta subunit	Q09RD5
675	1.57	0.0379	48.06	54.44	6.42	5.51	17	291	100	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	Q8MGI7
Biotic and abiotic stress											
1192	12.01	0.0273	26.37	27.21	9.3	6.16	8	146	100	Late embryogenesis abundant protein (AtECP63) (LEA)	Q9SKP0
269	7.5	5.07E-03	34.79	108.95	5.95	5.31	9	137	100	late embryogenesis abundant protein (LEA), putative	Q9LF88
268	6.58	0.0181	34.79	108.99	5.95	5.28	11	96	99.99	late embryogenesis abundant protein (LEA), putative	Q9LF88
1094	5.03	4.27E-03	29.99	32.11	6.26	5.58	11	293	100	Ferritin-1, chloroplastic	Q39101
931	4.36	7.58E-03	29.96	42.3	5.71	4.65	3	102	99.99	hydroxyproline-rich glycoprotein family protein	Q945P1
1370	4.26	8.68E-03	28.25	18.33	8.32	5.38	17	423	100	late embryogenesis abundant protein (LEA), putative	Q1IYB6
503	4.24	0.0146	76.76	73.42	5.19	5.03	16	207	100	70 kDa heat shock protein (HSP70)	Q9LHA8
413	3.78	0.0164	86.02	85.86	5.32	4.97	20	211	100	Heat shock protein 81-2 (HSP81-2)	P55737
1180	3.65	0.0181	24.36	27.82	8.33	6.09	11	185	100	1-cysteine peroxiredoxin PER1	O04005
669	3.43	0.0128	32.61	55.53	6.06	5.36	14	171	100	UTP--glucose-1-phosphate uridylyltransferase, putative	P57751
1361	2.91	0.0189	28.25	18.48	8.32	5.06	11	170	100	late embryogenesis abundant protein (LEA), putative	Q1IYB6

493	2.87	6.65E-03	76.76	73.95	5.19	4.98	25	427	100	70 kDa heat shock protein (HSP70)	Q9LHA8
1528	2.72	0.0116	16.31	14.78	9.78	5.83	7	128	100	Glutaredoxin-C2, putative	Q9FNE2
421	2.62	0.0136	85.76	86.02	5.18	4.95	26	409	100	Heat shock protein 81-2 (HSP81-2)	P55737
528	2.49	0.0402	72.03	71.34	9.31	5.61	17	191	100	stress-inducible protein, putative	Q9LHA8
254	2.35	0.0384	34.79	109.94	5.95	5.23	13	196	100	late embryogenesis abundant protein (LEA), putative	Q9LF88
771	2.29	0.0157	51.11	49.42	6.35	5.39	16	373	100	Monodehydoascorbate reductase 1	Q9LFA3
1246	2.18	0.0221	25.73	25.73	6.11	4.71	4	116	100	Germin-like protein subfamily 2 member 1	P94014
651	2.16	0.0279	57.01	57	6.36	4.74	12	144	100	Protein disulfide-isomerase	Q9SRG3
844	2.1	0.0252	33.69	46.24	6.45	5.41	13	300	100	Phosphoribulokinase, chloroplastic	P25697
1174	2.08	0.0128	30.5	31.23	6.44	5.07	16	331	100	rubber elongation factor (REF) family protein	Q9MA63
594	1.98	0.0238	49.18	61.88	5.56	5.21	15	426	100	Chaperone 60 beta	B9DHQ8
506	1.97	0.0208	76.76	74.44	5.19	5.08	24	596	100	70 kDa heat shock protein (HSP70)	Q9LHA8
504	1.93	0.03	71.36	74.61	5.31	5.19	15	161	100	70 kDa heat shock protein (HSP70)	P26413
1258	1.92	0.0162	31.79	24.36	8.71	5.92	12	148	100	Oxidoreductase	Q9LPL8
768	1.85	0.0128	51.11	49.6	6.35	5.31	21	441	100	Probable monodehydroascorbate reductase, cytoplasmic isoform 3	Q9LFA3
1218	1.84	0.013	33.85	25.54	7.19	5.06	13	552	100	2-cys peroxiredoxin, chloroplast	Q96291
981	1.78	0.0221	33.51	40.04	5.34	4.33	10	193	100	late embryogenesis abundant protein (LEA), putative	Q9LJ97
408	1.69	0.0391	85.76	85.76	5.18	5.00	13	134	100	Heat shock protein 81-2 (HSP81-2)	P55737
985	1.69	0.0128	38.88	43.55	5.2	4.98	13	272	100	dessication-related protein, putative	Q8LAU8
923	1.67	0.024	39.73	42.1	5.54	5.10	10	97	99.99	epoxide hydrolase, putative	Q9SD45

643	1.63	0.0128	57.01	56.48	6.36	4.79	8	164	100	Protein disulfide-isomerase	Q9SRG3
946	1.61	0.0221	40.05	41.25	5.46	4.87	16	386	100	late embryogenesis abundant protein (LEA), putative	O80576
502	1.59	0.0221	49.39	73.59	6.04	5.25	11	192	100	70 kDa heat shock protein (HSP70)	Q9LHA8
1211	1.58	0.0355	33.85	25.71	7.19	4.93	14	475	100	2-cys peroxiredoxin, chloroplast	Q96291
1321	1.57	0.024	28.64	28.09	9.07	4.83	13	253	100	peroxiredoxin type 2,chloroplastic, putative	Q949U7
488	1.54	0.0206	76.76	74.42	5.19	4.95	19	387	100	70 kDa heat shock protein (HSP70)	Q9LHA8
Carbon metabolism											
805	5.62	5.07E-03	56.64	48.38	9.82	5.59	15	288	100	Phosphoglycerate kinase	Q9LD57
1011	3.79	0.0132	42.11	39.75	9.1	5.91	15	296	100	Glucose and ribitol dehydrogenase homolog 1	Q9FZ42
793	2.9	0.013	58.69	52.52	10.96	5.51	7	267	100	Phosphoglycerate kinase	Q9LD57
859	2.78	0.0157	44.84	45.17	8.52	5.04	17	429	100	Sedoheptulose-1,7-bisphosphatase, chloroplastic	P46283
1166	2.51	5.07E-03	40.64	27.68	7.53	5.41	17	254	100	Triosephosphate isomerase, chloroplastic	Q9SKP6
967	2.45	0.0128	42.11	44.04	9.1	5.98	17	343	100	Glucose and ribitol dehydrogenase homolog 1	Q9FZ42
957	2.27	0.0165	49.64	42.59	9.22	5.57	13	457	100	fructose-bisphosphate aldolase, putative	Q9SJU4
787	2.18	0.021	58.69	48.54	10.96	5.35	9	110	100	Phosphoglycerate kinase	Q9LD57
913	1.75	0.0466	43.13	44.04	9.11	5.97	13	216	100	fructose-bisphosphate aldolase, putative	Q9SJQ9
884	1.67	0.0261	32.75	44.8	9.07	5.79	8	87	99.95	Malate dehydrogenase, cytoplasmic 2, putative	P57106
1036	1.58	0.021	53.65	36.64	8.53	5.39	11	135	100	lactoylglutathione lyase, putative	Q8W593
781	1.5	0.0206	56.69	50.14	6.69	6.15	15	240	100	Phosphoglycerate kinase (PGK1)	Q9SAJ4

Amino acid metabolism

1344	6.75	4.27E-03	22.37	19.37	8.31	4.69	4	91	99.98	Glycine decarboxylase complex H-protein, mitochondrial, putative	O82179
731	4.16	0.0179	48.09	51.07	9.18	5.32	10	171	100	Cysteine synthase, mitochondrial	Q43725
753	3.03	0.0216	52.99	51.11	6.17	5.78	12	198	100	Putative aminotransferase (POP2)	Q94CE5
881	2.9	9.00E-03	54.99	43.55	7.1	4.98	13	438	100	Cysteine synthase, mitochondrial	Q43725
557	2.68	0.021	46.19	67.98	9.67	5.85	9	98	99.99	histidine decarboxylase, putative	Q9MA74
728	2.11	0.021	52.36	55	9.42	4.77	12	295	100	N-acyl-L-amino-acid amidohydrolase, putative	Q9SZM2
799	1.89	0.0332	46.09	48.72	5.41	5.82	12	248	100	Elongation factor Tu, chloroplastic	P17745
586	1.57	0.0391	79.02	60.22	7.18	5.07	15	263	100	Glutamate decarboxylase 1	Q42521

Other metabolism

470	10.21	0.0157	49.49	84.01	9.19	5.90	10	84	99.9	alcohol oxidase-related	O65709
463	8.51	4.27E-03	49.49	84.88	9.19	5.84	9	83	99.89	alcohol oxidase-related	O65709
476	6.8	7.08E-03	49.49	84	9.19	5.06	12	183	100	alcohol oxidase-related	O65709
1430	4.34	0.016	20.56	17.32	5.74	5.97	9	133	100	Cyanate hydratase	O22683
806	4.31	5.07E-03	58.22	48.99	6.22	5.84	15	284	100	GDP-mannose 3,5-epimerase	Q93VR3
802	2.85	0.0128	58.22	48.77	6.22	5.73	14	366	100	GDP-mannose 3,5-epimerase	Q93VR3
982	2.33	8.81E-03	40.02	40.1	5.9	4.62	12	329	100	Probable plastid-lipid-associated protein 2, chloroplastic	O49629

658	1.63	0.0264	31.14	56.58	7.75	4.76	10	195	100	Tubulin beta-8 chain	P29516
Regulation											
1028	2.62	9.20E-03	43.23	37.09	5.63	4.66	9	296	100	14-3-3-like protein GF14 nu	Q96299
1044	1.82	0.0162	36.47	36.4	5.42	4.72	13	359	100	14-3-3-like protein GF14 nu	Q96300
1092	1.68	9.05E-03	35.11	32.18	5.16	4.69	14	482	100	14-3-3-like protein GF14 nu	Q96300
Other proteins											
734	2.91	5.07E-03	61.58	51.66	6.78	5.47	27	600	100	eukaryotic translation initiation factor 4A-1, putative	A8MRZ7
1081	2.5	7.58E-03	39.1	33.45	6.23	4.56	12	188	100	Elongation factor 1-delta 1	P48006
1313	1.78	0.0285	29.56	20.72	8.74	5.68	10	123	100	Peptidyl-prolyl cis-trans isomerase. chloroplastic	Q9ASS6
825	1.77	4.27E-03	45.54	47.1	6.05	5.35	14	314	100	Putative EF-hand containing protein	Q9T0I9
370	1.76	0.0258	93.92	96.4	5.8	5.89	16	103	99.95	similar to elongation factor 2	
1038	1.66	0.0391	43.74	35.84	9.2	5.67	14	282	100	Probable membrane-associated 30 kDa protein, chloroplastic	O80796
996	1.57	0.0273	43.25	38.57	6.17	4.42	10	227	100	31 kDa ribonucleoprotein, chloroplastic	Q04836
Unknown proteins											
1234	4.02	4.27E-03	30.16	26.14	6.84	4.58	13	236	100	hypothetical protein	
1363	2.97	7.34E-03	18.14	19.36	6.17	5.77	11	179	100	hypothetical protein	
1228	2.5	0.0273	31.5	25.22	8.63	5.46	13	566	100	hypothetical protein	

1476	2.39	0.0341	15.7	21.48	7.1	5.06	6	104	100	no hits found
584	2.14	0.0352	64.96	65.79	8.86	5.79	9	83	94.91	hypothetical protein
1224	1.97	0.0379	31.5	25.29	8.63	5.29	11	577	100	hypothetical protein
1394	1.89	0.0344	30.56	18.2	10.06	5.72	7	145	100	hypothetical protein
1624	1.89	4.27E-03	46.01	95.45	5.47	4.79	5	97	99.99	hypothetical protein
1376	1.78	7.58E-03	17.33	17.79	5.94	5.47	9	177	100	hypothetical protein
926	1.63	0.0141	29.85	47	5.15	5.12	7	335	100	hypothetical protein

Table 4. Functional categorization of proteins with higher abundance in the dry state compared with the hydrated state that were statistically different between dry and hydrated states with average ratios less than 1.5.

SPP	Av. Ratio	Pval	Th Mr (Kd)	Exp Mr (Kd)	Th p *I*	Exp p *I*	Pep	MOWSE Score	C.I.%	Name	Uniprot
Energy											
674	1.38	0.0483	474.52	56.16	6.18	5.46	12	192	100	ribulose-1,5-bisphosphate carboxylase/oxygenase	Q8HT76
Amino acid metabolism											
590	1.32	0.031	790.19	61.8	7.18	5.01	9	92	99.985	Glutamate decarboxylase 1	Q42521
Unkown proteins											
1393	1.48	0.0351	258.62	18.59	9.3	5.91	7	96	99.994	no hits found	

Table 5. Functional categorization of proteins with higher abundance in the hydrated state compared with the dry state that were statistically different between dry and hydrated states with average ratios higher than 1.5.

SPP	Av. Ratio	Pval	Th Mr	Exp Mr	Th p I	Exp p I	Pep	MOWSE Score	C.I.%	Name	Uniprot
Energy											
1462	-2.13	4.27E-03	157.16	16	6.93	4.83	8	150	100	V-type proton ATPase subunit F	Q9ZQX4
1255	-1.94	0.039	284.66	27.72	5.69	4.98	4	140	100	light-harvesting chlorophyll a/b-binding protein of photosystem II	O80388
1564	-1.83	0.0251	357.58	13.58	5.7	4.70	5	182	100	Light harvesting chlorophyll a/b-binding protein	Q9XF87
1511	-1.72	0.0409	222.37	14.85	10.17	4.81	4	271	100	Photosystem I reaction center subunit VI-2, chloroplastic	Q9SUI6
1422	-1.63	0.036	222.37	21.11	10.17	4.84	6	329	100	Photosystem I reaction center subunit VI-2, chloroplastic	Q9SUI6
Biotic and abiotic stress											
990	-2.62	9.05E-03	459.07	38.75	7.74	5.32	12	172	100	Thiazole biosynthetic enzyme, chloroplastic	Q38814
1183	-1.59	0.0314	268.76	26.87	5.93	5.76	8	154	100	glutathione transferase	Q9ZRW8
Carbon metabolism											
1262	-3.25	0.0195	304.36	23.53	8.01	5.29	11	103	99.999	lactoylglutathione lyase, putative	Q2V4P7
Amino acid metabolism											
1423	-1.92	0.0363	167.86	22.0	9.64	5.25	8	125	100	Nucleoside diphosphate kinase 1	P39207

Other Metabolism											
1465	-1.97	0.0351	165.88	16.3	4.8	4.78	9	105	99.969	serine/threonine kinase-like	
Other proteins											
878	-2.65	0.0392	352.49	44.71	4.58	4.25	7	125	100	outer membrane protein C [Salmonella enterica subsp. enterica serovar Paratyphi A str. AKU_12601]	
1111	-1.86	0.0402	299.46	30.34	5.54	4.58	7	221	100	Eukaryotic translation initiation factor 6 (EIF-6)-like protein	Q9M060
Unknown proteins											
223	-2.92	5.07E-03	657.91	108.52	4.75	4.63	18	163	100	hypothetical protein	
222	-2.54	0.0151	657.91	108.83	4.75	4.59	17	119	99.999	hypothetical protein	
1045	-1.36	0.0238	362.18	34.15	8.79	5.01	4	179	100	hypothetical protein	

Table 6. Functional categorization of proteins with higher abundance in the hydrated state compared with the dry state that were statistically different between dry and hydrated states with average ratios of less than 1.5.

SPP	Av. Ratio	Pval	Th Mr	Exp Mr	Th p *I*	Exp p *I*	Pep	MOWSE Score	C.I.%	Name	Uniprot
Energy											
822	-1.46	0.0353	59.34	46.97	9.19	4.91	14	113	100	RUBISCO activase (RCA), putative	Q3EBJ5

1405	-1.46	0.0385	26.48	17.56	8.94	5.34	6	235	100	cytochrome c oxidase family protein, putative	A8MRD7
1207	-1.25	0.0256	35.76	36.4	5.7	4.64	6	194	100	Light harvesting chlorophyll a/b-binding protein	Q9XF87
Carbon metabolism											
1014	-1.34	0.0224	3.645	37.33	8.53	5.01	12	113	100	lactoylglutathione lyase, putative	Q8W593
Other metabolism											
1181	-1.44	0.0183	33.67	26.81	9.72	5.57	7	155	100	flavin reductase-related	Q8H124
Unknown proteins											
1045	-1.36	0.0238	36.22	34.15	8.79	5.01	4	179	100	hypothetical protein	
989	-1.29	0.0466	38.88	38.9	5.2	4.79	13	565	100	hypothetical protein	
979	-1.24	0.0196	30.63	39.29	5.02	5.07	19	604	100	hypothetical protein	

4 Discussion

4.1 Energy

Carbonic anhydrase (CA) was among the proteins that were detected and categorized as energy related and high in abundance in dry state (spots 1135, 1095), this protein is related to the reversible hydration of carbon dioxide. The expression of chloroplastic CA from tobacco plants has shown to play a role in the defense response of the plant against diseases (Forouhar et al., 2005; Slaymaker et al., 2002). Other enzyme with high abundance in dry state was RUBISCO (Spots 1078, 702, 684, 70, 683, 617, 672 and 675), which catalyzes the carboxylation of D-ribulose 1,5-bisphosphate, and the fragmentation of the pentose substrate in the photorespiration process. In the dry state of *S. lepidophylla*, there is no carbon dioxide uptake (Eickmeier, 1979), therefore, RUBISCO is most likely not active in the dry state, however, enzymatic essays performed by Bergtrom et al. (1982) showed that RUBISCO extracted from dry plants has the potential to perform its catabolic activity. Due to the lack of CO_2 exchange during the dry state in *S. lepidophylla,* the presence of RUBISCO in the dry state could serve as a carbon reserve. Alternatively, the plant can perform photosynthesis until the last stages of dehydration and then stored RUBISCO in order to prepare for the recovery process during rehydration.

Another protein present with high abundance in the dry and hydrated state includes RUBISCO activase (RCA) (Spots 756, 832, and 822). This protein is required for the activation of RUBISCO. Increase in temperature tends to decrease RUBISCO activity

(Kurek et al., 2007). The reduction in the RUBISCO activation when temperature is slightly above optimum (30°C) is related to the inhibition of RCA, therefore, thermostable RCA can improve photosynthesis rates at elevated temperatures (Kurek et al., 2007). Salvucci (2008) proposed that RCA associated with chaperone 60 beta during heat stress improves thermostability of RCA. Considering that *S. lepidophylla* is native of desert areas, the presence of RCA and chaperone 60 beta (Spot 594) in the dry state could imply that this RCA is less susceptible to degradation at higher temperatures or under dehydration conditions allowing RUBISCO to function. In addition, there could be other processes controlling the activation of RUBISCO at high temperature aside from the presence of RCA (Yamori and Caemmerer, 2009).

4.2 Biotic and abiotic stress

Late Embryogenesis Abundant (LEA) proteins (Spots 1192, 269, 268, 1370, 1361, 254, 981 and 946) showed increased relative abundance in the dry state in *S. lepidophylla*. These kind of proteins have been categorized in six families depending on their sequence, among those six families groups 3 and 4 LEA proteins tend to accumulate during the process of dehydration and contribute to the cytoplasm vitrification that helps maintain the integrity of the cell and membranes (Battaglia et al., 2008; Shimizu et al., 2010). In addition, Group 3 LEA proteins tend to accumulate in the latter stages of seed maturation and in the vegetative state when the plant goes through periods of dehydration. The other families of LEA proteins are related to several kinds of stresses like cold, heat, UV light, drought, salinity and wounding (Battaglia et al., 2008)The high abundance of LEA

proteins in the dry state of *S. lepidophylla* can contribute to the cell and membranes integrity due to the scarcity of water in the dry state.

Other proteins that were expressed in higher abundance in the dry state than in hydrated state were heat shock proteins (HSPs) (Spots 503, 493, 421, 504, 506, 408, 502 and 488). These proteins have a role in protein stability and folding (Neilson et al., 2010) under normal and stressful conditions (Wang et al., 2004). The HSPs proteins have been divided into five families, the HSP70, chaperonins (GroEL and HSP60), the HSP90, the HSP100and the small HSP families (Wang et al., 2004). Among those families, under dry conditions HSP70 (Spots 503, 493, 504, 506, 502, and 488), HSP60 (594) and HSP80-1 (421, and 408) were present in high abundance. The HSP70 protein family play stress protective roles in diverse processes such as protein denaturation, fungal infections, (Duan et al., 2010) prevention of protein aggregation, assist protein refolding, import and translocation processes and facilitation of photolytic degradation of unstable proteins (Neilson et al., 2010; Timperio et al., 2008; Wang et al., 2004). The role of HSP60 protein family has not been fully characterized, it is considered that they play a role in assisting plastid proteins like RUBISCO (Salvucci, 2008; Wang et al., 2004) and Cpn60 alpha mutants in *Arabidopsis* present defects in chloroplast development (Wang et al., 2004) The high abundance of HSPs 70 and 60 families in dry state could indicate that in *S. lepidophylla* these proteins are necessary to prevent protein aggregation or denaturation, or help fold denatured proteins when water is available for rehydration and they could play a vital role in this plant heat acclimation.

4.3 Carbon metabolism

Several proteins found in high abundance in dry state play a role in carbon metabolism and carbohydrate biosynthesis, such Glucose and ribitol dehydrogenase homolog 1 (Spots 967 and 1011), which belongs to short alcohol dehydrogenases. The over-expression of this protein in yeast mutants resulted in enhanced ability to grow on salt media a result that was related to the possible accumulation of sugars with osmoprotective functions because osmotic shock stimulates the glycolitic pathway (Witzel et al., 2010). If the production of sugars is enhanced with osmotic shock due to drought stress or salinity, these compound compounds could act as osmoprotectants, and contribute to cytosolic vitrification (Scott, 2000; Zentella et al., 1999) to maintain cell and membranes integrity in the dry state of *S. lepidophylla*.

4.4 Amino acid metabolism

The proteins detected that have functional roles in amino acid metabolism were sulfur-related (Spot 731) or degradation of amino acids such as glycine (Spot1344). In addition a protein involved in gamma-aminobutyric acid (GABA) metabolism was found, glutamate decarboxylase (Spot 586), which acts on L-glutamate, to produce 4-aminobutanoate and carbon dioxide. GABA is a metabolite that is present in almost all prokaryotic and eukaryotic organism and its accumulation is associated with response to stress (Shelp et al., 2009). In plants, GABA accumulates as a response to several biotic and abiotic stresses that might damage membranes like wounding, freezing, heat stress (Allan et al., 2009) and salt stress, but its exact molecular function has not been found yet (Renault et al., 2010). Therefore, it could be possible that in *S. lepidophylla,* the

accumulation of glutamate decarboxylase is related to a defense mechanism of the plant to maintain the integrity of membranes associated to GABA accumulation due to heat or salt stresses.

4.5 Regulation

The only protein found associated with signaling and protein regulation in higher abundance in dry state was the 14-3-3-like protein GF14 nu (Spots 1028, 1044 and 1092). 14-3-3 protein in *Arabidopsis* are associated with disease resistance by acting as a positive regulator of RPW8 gene (Duan et al., 2010). In addition, 14-3-3 protein has been related to several signal transduction cascades in different kind of stresses in *P. patens* (Wang et al., 2009a).

4.6 Other metabolisms

GDP-mannose 3,5-epimerase is the enzyme that precedes the committed step in the biosynthesis of vitamin C (L-ascorbate), which catalyzes the reaction from GDP-mannose to GDP-L-galactose (Wolucka and Montagu, 2003). Ascorbate is a metabolite that acts as an antioxidant and can associate to other compounds to protect plants from other oxidative damages, photosynthesis and pollutants (Smirnoff, 1996). The presence of GDP-mannose 3,5-epimerase in higher abundance in dry state could indicate that the plant was producing high amount of L-ascorbate as it was suffering from drought and oxidative stress in the dehydration process. In addition, it has been reported an association between HSP70 and GDP-mannose 3,5-epimerase, which could be involved in the correct folding of the enzyme during stress (Ingle et al., 2007; Wolucka and

Montagu, 2003). Since both proteins were found to be in higher abundance in dry state, there could be an association between HSP70 and GDP-mannose 3,5-epimerase that confers stability to the enzyme in a stressful environment that can help in the production of L-ascorbate.

4.6 Conclusions

This comparative proteomic study of *S. lepidophylla* dry and hydrated states, detected 1,700 spots of which 184 spots were successfully sequenced. Of those 184 spots, 130 had an ANOVA value < 0.05 of which 109 had a higher abundance in the dry state with an average ratio of 1.5 or higher, and 3 with an average ratio between 1.3 and 1.5. Of the 130 spots, 15 had a lower abundance in the dry state with an average ratio of -1.5 or higher, and 8 with an average ratio between -1.2 and -1.5. Some of the proteins with a higher abundance in dry state were Rubisco, RCA, LEA proteins, HSPs, 14-3-3 proteins and protein with functions related to photosynthesis, energy storage, control of RUBISCO, cytoplasm vitrificaton, protection of membrane integrity, protein folding aid, and defense response.

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