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Research Article

The Proteomic Changes in *Cynara Cardunculus* L. var. *altilis* DC Following the Etiolation Phenomena Using *De Novo* Sequence Analysis

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Etiolation of vegetables is a complex phenomenon implying qualitative and quantitative protein changes. The 2-DE protein profile of green and etiolated fleshy stalk samples has shown great differences in the 4–7 pH and 10 to 250 kDa mass range. Currently, a shortage of *Cynara* DNA or protein sequences has required the use of *de novo* sequencing and BLAST similarity searches for protein identification. Highlighted herein is an application of proteomics to the identification of proteins, which in a great part matched those from the *Viridiplantae* order. Quantitative evaluation, statistical analyses, and MALDI-TOF MS characterization of the resolved spots in green and etiolated samples of *C. cardunculus* enabled us to identify 46 out of 60 spots, containing 21 spots included conserved *Viridiplantae domains.* The etiolation process is discussed in light of the identification of some proteins involved in specific biochemical and metabolic pathways.

1. Introduction

In dark-grown angiosperm plants, chlorophyll biosynthesis is arrested at the Pchlide stage (etiolation) due to the deficiency of a light-requiring oxidoreductase enzyme which naturally converts Pchlide to chlorophylls. In parallel, the natural content of fibre, bitter, and aromatic compounds declines in vegetables [1–4]. By and large, the edible portion of plants corresponds to etiolated foliated rosettes or young stalks of biennials or perennials. Transient etiolation of leaves and other tissues takes place naturally during spring development under light-deficit. The same phenomena can be reproduced by transferring plants to a dark chamber at optimal temperature for growth. During etiolation, various biochemical and anatomical processes occur, implying mainly an increased synthesis and accumulation of plastidial proteins. Little information had thus far been available on the expressed plant proteins during etiolation and subsequent deetiolation. A more detailed characterization of vegetable proteins using proteomics is needed for a more comprehensive view of the physiological changes associated to the etiolation process. A deeper understanding of the cellular events is highly desirable also in view of manipulations aimed to improve sensorial, nutritional, and shelf life features.

The cultivated cardoon (*Cynara cardunculus* L. var. *altilis* DC) is one of the vegetables most subjected to etiolation. *Cynara cardunculus* L. is a diploid ($2n = 34$) out crossing perennial species native to the Mediterranean basin and comprising the globe artichoke (var. *scolymus* L.), the cultivated cardoon (var. *altilis* DC), and the wild cardoon (var. *sylvestris* (Lamk) Fiori). *Cynara cardunculus* var. *sylvestris* is considered the wild ancestor of the globe artichoke and cultivated cardoon [5–7]. The latter has been considered one of the most important perennial herbaceous cultivated plants mainly for the blanched leaf petioles, regarded in the Mediterranean area as a delicious speciality. Interest towards

this crop is growing in European countries, especially in Spain, France, and Italy. In Portugal, *Cynara cardunculus* L. cardoon is traditionally used as a source of two aspartic proteinases, Cardosins A and B, for milk clotting in the manufacturing of cheese [8].

Mass spectrometry- (MS-) based proteomics has been used extensively to explore the proteomes of various cells and organisms. MS technology is now being applied to protein identification as it offers high sensitivity and, when coupled with liquid chromatography, reduces sample complexity and increases high-throughput capability [9].

Recently, high-resolution tandem mass spectrometers have enhanced robustness of peptide identification [10] based on matches between the measured mass of the peptides resulting from the protein digestion with high specificity proteases and the corresponding fragments in the protein databases. This approach is unable, however, to deal with proteomes or genomes that have not yet been sequenced.

In such cases, *de novo* sequence determination and sequence similarity searches provide alternative approaches to address the protein identification. Several software tools have been developed to deduce an amino acid sequence from an MS/MS spectrum [11, 12]. Furthermore, database search algorithms like BLAST [13, 14], recently optimised to deal with the sequence query length typical of mass spectrometric data [15, 16], have been utilized in cross-species identification of unknown proteins. However, the quality of tandem MS data and both *de novo* sequence interpretation and similarity search algorithms is critical, possibly leading to false positive or false negative matches. Such approaches have been successfully applied to protein identification from various organisms with unsequenced genomes [17, 18], including that of vegetables [19–22]. However, no rule has been established indicating the length, the number of fragmented peptides, or the de novo sequencing accuracy for a positive identification of homologous proteins. Moreover, the sequence percentage required for the identification of homologous proteins is also undetermined. Simulation has suggested that more than 80% of mammalian proteins could be positively identified on the basis of similarity between orthologous proteins of different species [23]. Therefore, for a successful characterization of a proteome by mass spectrometry, availability of the relative genome may not be a prerequisite.

On the basis of this background, the main objective of this study was to investigate the changes of the proteins of *Cynara cardunculus* L. var. in relation to the etiolation process. Therefore, we compared global protein expression profiles of green and etiolated fleshy stalks of cultivated cardoon.

2. Materials and Methods

2.1. Plant Materials. Green and etiolated fleshy stalks of cultivated cardoon (*Cynara cardunculus* L. var. *altilis* DC) were used for this study. The system was realized in the Agricultural Company situated near to San Nicola Manfredi in Benevento district (Campania region) in March with direct dwell seeds in distant rows with 80 cm between each plant in plots of 3×3 meters. They have placed three seeds per hole, which leads to a single plant. In the period of May-June, the plants were in the rosette phase. The plants reached their complete development at the end of summer-beginning of autumn (August-September). At the end of autumn (October), some green fleshly stalks were collected from three adjacent plants, quickly washed in sterile distilled water, freeze-dried, and stored at -80°C until analysis. Continuous dark for 25/30 days provoked plant etiolation as the leaves became more tender, white, and sweet as well. At the end of December, the etiolated fleshly stalks were collected and quickly washed in sterile distilled water, freeze-dried, and stored at -80°C until analysis.

2.2. Protein Extraction. Total proteins were extracted by a phenol-based method described by Saravanan and Rose [24]. The fleshy stalks were finely powdered in liquid nitrogen, using a mortar, and suspended in 7.5 mL of extraction buffer (700 mM sucrose, 500 mM Tris-HCl, pH 8.0, 50 mM EDTA, 100 mM KCl, 2% v/v β-mercaptoethanol, and 1 mM phenylmethylsulphonylfluoride), by vortexing for 15 minutes at 4°C. After addition of an equal volume of phenol saturated 500 mM Tris-HCl, pH 7.5, the mixture was vortexed extensively for 10 minutes and then centrifuged at 10000 g for 15 minutes at 4°C. The upper phenol phase was removed and extracted twice with the extraction buffer. Proteins were precipitated from the phenol phase by the addition of five volumes of saturated ammonium acetate in methanol, overnight at -20°C. Precipitated proteins were centrifuged at 10000 g for 30 minutes.

2.3. 2-DE. Protein pellets were washed with ice-cold methanol (once) and ice-cold acetone (three times) dried and solved in IEF buffer (9 M urea, 4% w/v CHAPS, 20 mM DTT and 1% w/v ampholyte pH 3–10) (Bio-Rad, Hercules, CA, USA). Protein concentration was quantified using the BioRad protein assay using BSA as a standard. IPG strips (18-cm pH 4–7 Bio-Rad ReadyStrip), rehydratated overnight with 315 μL of IEF buffer containing 400 μg of total proteins. Proteins were focussed using a Protean IEF Cell (Bio Rad) at 12°C, applying 250 V (1 h), 1000 V (5 h), and 8000 V for a total of 52 kVh. Prior to the second dimension, the gel strips were reduced with 2% w/v DTT in 6 M urea, 30% w/v glycerol, 2% w/v SDS, 50 mM Tris-HCl pH 8.8, containing 0.01% w/v bromophenol blue for 20 minutes, followed by 20 minutes in the same buffer containing 2.5% w/v iodoacetamide. Electrophoresis in the second dimension was carried out using a Protean apparatus (BioRad) and 12% polyacrylamide gels (18 cm $\times$ 24 cm $\times$ 1 mM) in 25 mM Tris (pH 8.3), 1.92 M glycine, and 1% w/v SDS, with 70 V (l35 mA) being applied for 16 h. Each sample was run in triplicate. Protein spots were annotated only if detectable in all gels [25].

2.4. Image Acquisition and Analysis. 2-DE gels were stained with colloidal Coomassie G250 and scanned using a GS-800 calibrated densitometer (Bio-Rad). Image analysis was

performed using the PDQuest software (Bio-Rad). Spot detection and matching between gels were performed automatically, followed by manual verification. After normalization of the spot densities against the whole-gel densities, the percentage volume of each spot was averaged for six different (three replicates of two samples) gels and the Student's t-test analysis ($P < .01$) was performed to find out statistically significant protein fold changes associated to etiolation. A twofold change in normalized spot densities was considered indicative of a differentially expressed component.

2.5. In-Gel Tryptic Digestion. Spots in the Coomassie Brilliant Blue G-250-stained 2-DE gel were excised manually and before being subjected to in-gel trypsinolysis as previously reported by Mamone et al. [26], reduced (10 mM DTT in 100 mM NH_4HCO_3 allowing to react for 45 min, at 55°C) and alkylated with 55 mM iodoacetamide in 100 mM NH_4HCO_3 for 30 min at 25°C, in the dark. The resulting tryptic digest was dissolved in 40 μL of acetonitrile and 5% formic acid solution (1 : 1 v/v) and then the sample concentrated with vacuum centrifuge on medium heat to one-tenth initial volume for mass spectrometric analysis.

2.6. Reverse Phase Capillary Chromatography. Reverse phase capillary chromatography coupled with quadrupole time-of-flight tandem mass spectrometry was carried out using a Waters CapLC ternary pump system connected through a stream-select valve module to the nano-Z-spray source of the mass spectrometer. The sample was injected into the system through the CapLC autosampler. Solvent A and C consisted of water with 0.1% TFA, and solvent B consisted of acetonitrile with 0.1% TFA.

The protein digest was preconcentrated and desalted on a precolumn cartridge packed with Atlantis dC18 resin at flow rate of 20 μL/min-1 over 3 minutes. After switching to pumps A and B, a linear gradient from 5 to 70% of B over 45 min at a flow-rate of 300 nL min-1 (2% B/min) was applied to the precolumn cartridge and then used to elute the sample from the Atlantis dC18 resin analytical column (15 cm $\times$ 75 μm i.d.).

2.7. Mass Spectrometry. All mass spectra were acquired using a quadrupole-TOF hybrid mass spectrometer Waters Q-TOF II equipped with a nano-Z-spray source operating in positive ion mode. The ionization conditions used included a capillary voltage of 2.6 kV, source temperature of 80°C, and no nebulising gas. External calibration was applied to all data using Glu1-Fibrinopeptide B (Sigma-Aldrich) MS/MS fragments over a mass range from 50 to 1600 m/z.

Survey scan acquisition was done on-line with capillary chromatographic separation; an initial TOF-MS scan was acquired over the mass range of 400–1600 m/z each second, with switching criteria for MS to MS/MS that included ion intensity (10 counts/s) and charge state (+2 to +4). MS/MS of precursor ion selected was acquired over the mass range of 50–1600 m/z. The collision energy used varied automatically according to the mass and charge state of the eluting peptides.

2.8. Nanoflow LC-ESI-MSMS. LC-ESI-MSMS analysis was performed using a Q-TOF Ultima mass spectrometer (Waters/Micromass UK Ltd., Manchester, UK) utilizing automated data-dependent acquisition. A nanoflow HPLC system (Waters CapLC (Waters/Micromass UK Ltd., Manchester, UK) was used for chromatographic separation of the peptide mixtures prior to MS detection. The peptides were concentrated and desalted on a precolumn cartridge packed with Atlantis dC18 resin (Waters CapLC, Waters/Micromass UK Ltd., Manchester, UK) and eluted at 200 nL/min by an increasing concentration of acetonitrile (2%/min gradient) onto an analytical column (15 cm $\times$ 75 μm i.d.) also packed with Atlantis dC18 resin. An MS-TOF survey spectrum was recorded for 1 s. The five most abundant ions present in the survey spectrum were automatically mass-selected and fragmented by collision-induced dissociation (4 s per MSMS spectrum). The MSMS data were converted to a pkl file format using the Mass-Lynx 3.5 ProteinLynx software, and the resulting pkl file was searched against the NCBInr protein sequence databases using the Waters ProteinLynx GlobalSERVER v2.2.5 (PLGS).

2.9. Protein Identification. Acquired raw data were deisotoped and converted to pkl files using Waters ProteinLynx GlobalSERVER v2.2.5 (PLGS). Database search was performed against the tryptic peptides of entire taxonomy order *Viridiplantae* of NCBI database using a peptide tolerance of 0.1 Da and fragment mass tolerance of 0.2 Da, allowing a maximum number of two missed cleavages. Carbamidomethylated cysteine was set as fixed modification, while oxidized methionine was set as variable modification. Automod algorithm was used to determine additional posttranslational modifications and/or single amino acid substitution. The MS/MS spectra of peptides not identified by the previous two algorithms were submitted to the PLGS "*de novo*" sequence algorithm and the sequences obtained were BLAST searched against of NCBI *Viridiplantae* database using the same software tool. Alignments parameters were adjusted to search for a short input sequence, that is, PAM30MS matrix and expected threshold set to 1000. Redundant database search and de novo sequence alignments match several homologous proteins of different species, making difficult to establish a scoring system at protein level. Data were filtered at peptide level allowing only peptide having at least three consecutive b- or y-fragment ions of an homologous protein sequence tag and a ladder score (percentage of y- and b-ions detected) greater than 40. Using a decoy database, generated randomising NCBI *Viridiplantae* database, the calculated false positive discovery rate was limited to 6.7%.

To detect the potential conserved domains included in the sequences deduced by mass spectrometry [27], the peptide sequences inferred were blast searched using the protein BLAST tools limiting the results to the accession numbers of homologous proteins having the higher number

of matches in each spot. Blast algorithm used was blastp with BLOSUM62 matrix and expected value set to 10.

3. Results and Discussion

3.1. Proteome Changes Observed during Etiolation. Proteins extracted from green and etiolated fleshy stalks of cultivated cardoon were separated by 2D-PAGE and spots were visualized by CBB staining. Analysis of spots on the 2-DE gel using the PDQuest 2D image analysis software (Bio-Rad) indicated approximately 200 proteins having an M_r less than 100 kDa and a *pI* in the range 4–7 (Figure 1). Taking into account the spot areas and their intensity, 60 protein spots were selected along each 2-DE gel for green and etiolated fleshy stalks. 21 out of 60 proteins were differentially expressed in green and etiolated fleshy stalks ($P < .01$) while 23 protein spots were found to be common to two types of fleshy stalks. By capillary LC-nano-ESI Q-TOF MS/MS sequence analysis 46 proteins (82%) were successfully identified. Among the sequences inferred from *Cynara cardunculus* proteins identified within 60 electrophoretic spots, ~ 71% tryptic peptides exactly matched the molecular mass and sequence of orthologous peptides occurring in the *Viridiplantae* database, the remaining differed by one or more amino acid residues inferred by the *de novo* sequence. The identification of conserved domains along the protein stretches using sequence similarity searches provides additional confidence on the function of the identified proteins although additional experiments are necessary to obtain ultimate confirmation. Occurrence of false positive and false negative matches may affect identification even using robust high-resolution techniques. Error may be caused essentially by sequencing errors derived from incomplete fragmentation and/or interpretation of spectra and/or erroneous sequence alignment. Taking into account these limitations, sequence similarity searches based on capillary LC coupled with nano-ESI Q-TOF data seem to represent a valid analytical tool capable of extending proteomic analysis to unsequenced genome organisms. As expected, Ile and Leu residues were indistinguishable in low-energy collision-induced dissociation spectra. Similarly, neither Gln nor Lys differing in 0.036 Da was discriminated, except for the C-terminal Lys in tryptic peptides.

The relative quantitative variations of the identified protein are shown in Table 1. Among the identified upregulated proteins, a large portion of the spots is represented by proteins involved in carbon/energy metabolism, defense from environmental/pathogen stresses, and oxidative stress control.

3.2. Proteins Associated with Carbon and Energy Metabolism. Different key enzymes of glycolysis related to starch catabolism are upregulated during etiolation. Their abundance likely reflected the pivotal role of this pathway that fuels substrates for respiration and organic acid and pigment synthesis from imported sugars during etiolation, as shown by cytosolic malate dehydrogenase (spot 16) that are implicated in the glycolyis and gluconeogenesis. Interestingly,

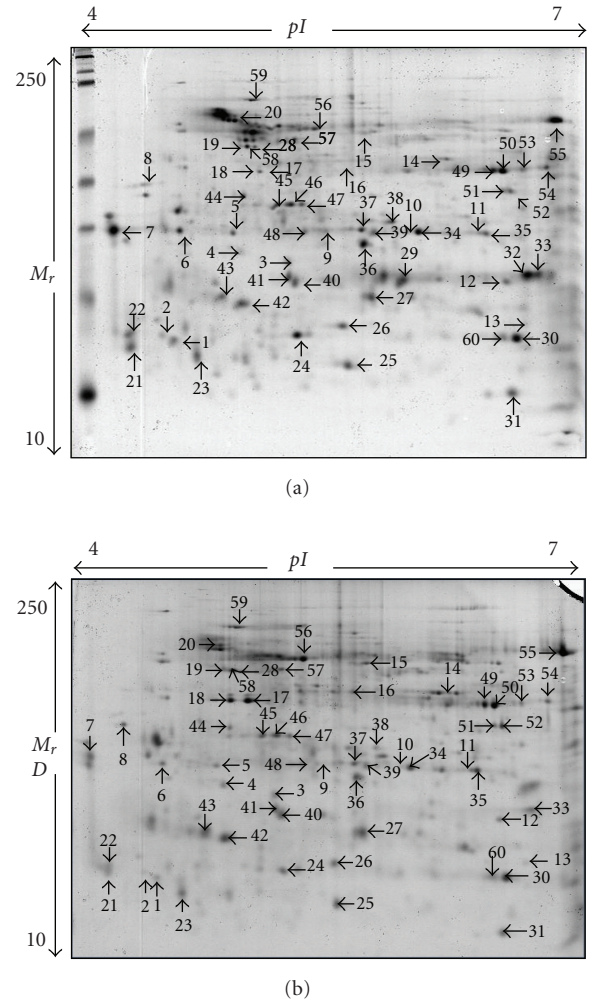

Figure 1: Representative 2-DE gels of total protein extracts from fleshy stalks of cultivated cardoon (*Cynara cardunculus* L. var. *altilis* DC). (a) Etiolated fleshy stalks; (b) green fleshy stalks. The horizontal bar indicates *pI* and the vertical one M_r. Spot numbering refers to Table 1. Gels were stained with colloidal CBB G-250.

levels of glycolytic enzymes on the whole decreased in etiolated fleshy stalks of cultivated cardoon (spot 10). In fact triosephosphate isomerase catalyses the reversible interconversion of the triose phosphate isomers dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate. It plays an important role in glycolysis and it is essential for efficient energy production. When the cytosolic triosephosphate accumulation inhibits the respiration, it triggers malate efflux from mitochondria and its oxidation. In fact, while starch is the predominant storage form in green fleshy stalks, etiolation is characterized by a decline in starch content, sucrose import, and accumulation of hexoses. Therefore, in photosynthetic tissues, mitochondria are obligatory partners of chloroplasts with respect to CO_2 light-dependent assimilation, due to their implication in the photorespiration pathway.

Spot 21 was identified as Acyl-CoA-binding protein (ACBP), homologous to that from *Panax ginseng*, and is a

Table 1: Constantly and differently expressed proteins in green and etiolated fleshy stalks classified according to functional categories. (a) Differentially expressed proteins and (b) constantly expressed proteins. Spot number, experimental *pI* and M_r values, protein description, accession number, hortologous organism, peptide sequences, and conserved domain are listed. Increasing/decreasing index (fold change) was calculated as the ratio of spot intensities (relative volumes) for green and etiolated fleshy stalks. Proteins were considered as differentially expressed when a relative fold change >2.0 or <0.5 was measured. (c) Abbreviated names of organisms: AT: *Arabidopsis thaliana*; CI × CE: *Cichorium intybus* × *Cichorium endivia*; CJ: *Cercidiphyllum japonicum*; CR: *Catharanthus roseus*; CS: *Cucumis sativus*; DC: *Daucus carota*; FB: *Flaveria bidentis*; GM: *Glycine max*; HA: *Helianthus annuus*; LS: *Lactuca sativa*; MT: *Medicago truncatula*; NP: *Narcissus pseudonarcissus*; NPl: *Nicotiana plumbaginifolia*; NS: *Nicotiana sylvestris*; NT: *Nicotiana tabacum*; OS: *Oryza sativa*; OSj: *Oryza sativa japonica*; OT: *Ostreococcus tauri*; PB: *Pimpinella brachycarpa*; PG: *Panax ginseng*; PS: *Pisum sativum*; PT: *Populus trichocarpa*; SA: *Sinapis alba*; SP: *Schizosaccharomyces pombe*; ST: *Solanum tuberosum*; TA: *Triticum aestivum*; TS: *Tsuga heterophylla*; VV: *Vitis vinifera*; ZH: *Zinnia elegans*.

Spot	*pI* /M_r (kDa)	Relative Fold change Green/Etiolated	Description	Accession number	Hortologous Organisms (c)	Exact Match sequence	"*de novo*" sequence	Conserved domains
Carbon\Nitrogen metabolism or energy								
(a) Differentially expressed proteins								
10	6.1/30	5.30	Triosephosphate isomerase cytosolic	P48493	LS	(K)VASPAQAQEVHAGLR(K) (K)VIAC$_m$VGETLEQR(E) (L)AYEPVWAIGTGK(V) (R)EAGTTM$_{ox}$EVVAAQTK(A) (R)IIYGGSVSGSNC$_m$K(E)	N(I/L)SADVAGDTR VASPA(Q/K)A(Q/K)EVYAE(I/L)R	cd00311
16	5.7/56	0.40	Malate dehydrogenase cytoplasmic	Q7XDC8	OSj	(E)FISTVQQR(G) (R)ALGQISEK(L)	(Q/K)G(I/L)(Q/K)(I/L)DEFSRARMD-A	
25	5.7/10	3.00	Vacuolar membrane ATPase subunit G	AAD54418.1	AT	—	R(I/L)E(Q/K)ETDE(Q/K)(I/L)AG(I-/L)(I/L)TR EAGAA(I/L)EA(Q/K)(Q/K)(I/L)VNA-AR (Q/K)(I/L)TETSGDSGANV(Q/K)R (I/L)SSDVVEM$_{ox}$(I/L)(I/L)K A(Q/K)MEADY(Q/K)R	
30	6.8/11	3.60	Nucleoside diphosphate kinase B	Q39839	GM	(-)MDEQTFIMIKPDGVQR(G)	M(I/L)WEG(Q/K)NVV(I/L)TGR(Q-/K)(I/L)(I/L)GATNPAASEPGT(I/L)R	cd04413
			Nucleoside diphosphate kinase B	P47920	FB	(E)IIGATNPAESAPGTIR(G) (E)IIGATNPAESAPGTIRGDFAI-DIGR(N) (E)PGTIR(G) (K)NVVTTGR(E) (L)VGEILGRFEK(K) (R)GDFAIDIGR(N) (R)GDFAIDIGRNVIHGSDAV-ESAKK(E) (R)GLVGEIIGRFEK(K) (R)GLVGEILGR(F) (R)NVIHGSDAVESAK(K) (R)NVIHGSDAVESAKK(E) (V)IHGSDAVESAKK(E)		

Table 1: Continued.

Spot	pI /M_r (kDa)	Relative Fold change Green/Etiolated	Description	Accession number	Hortologous Organisms (c)	Exact Match sequence	"*de novo*" sequence	Conserved domains
			Carbon\Nitrogen metabolism or energy					
			RNA binding translational regulation protein of the SUA5 family ISS	CAL50272.1	OT	(R)EGLLGR(I)		
21	4.3/11	0.08	Acyl CoA binding protein	BAB85987.1	PG	(K)QATVGPVNTSR(P) (K)TLPESSTTNENK(L) (K)LILYGL(Y)	G(I/L)(K/Q)EDFEEHAEK	cd00435
60	6.6/12	3.10	Nucleoside diphosphate kinase B	P47920	FB	(N)PAESAPGTIR(G) (V)IHGSDAVESAK(K) (A)TNPAESAPGTIR(G) (R)NVIHGSDAVESAK(K) (K)IIGATNPAESAPGTIR(G)		cd04413
36	5.8/27	2.40	Oxygen evolving enhancer protein 2, chloroplast	Q40407	NP	(K)EVEFPGQVLR(Y) (K)VDYLLGK(Q) (K)WNPSKEVEFPGQVLR(Y)	EATDFGPPEEM$_{ox}$(I/L)SK AYGEAANVFG(Q/K)PK GDDTAEHSHSAT(I/L)TATVSGGK	pfam01789
45	5.2/38	2.40	Oxygen-evolving enhancer protein 1, chloroplast	Q40459	NT	(R)GSSFLDPK(G) (K)RLTFDEIQSK(T)		
46	5.3/38	2.40	Oxygen evolving enhancer protein 1, chloroplast	P26320	ST	(R)GGSTGYDNAVALPAGGR(G) (R)LTFDEIQSK(T) (R)VPFLFTIK(Q) (S)SFLDPK(E) (T)YLEVK(G) (V)PFLFTIK(Q)	GDEEE(I/L)(I/L)(Q/K)EN(I/L)K	pfam01716
47	5.4/38	2.30	Oxygen evolving enhancer protein 1, chloroplast	P26320	ST	(R)VPFLFTIK(Q) (K)RLTFDEIQSK(T) (K)FC$_m$LEPTSFTVK(A)		

Table 1: Continued.

Spot	pI /M_r (kDa)	Relative Fold change Green/Etiolated	Description	Accession number	Hortologous Organisms (c)	Exact Match sequence	"*de novo*" sequence	Conserved domains
				Carbon\Nitrogen metabolism or energy				
				Oxidative stress				
38	6.0/32	3.10	Ascorbate peroxidase	AAF22246.1	PB	(K)EGLLQLPTDK(A) (K)TGGPFGTMR(L) (K)TGGPFGTM_{ox}R(L)	WGEVRVD(Q/K)EEPP(Q/K)NGR EEPP(I/L)EGR	
55	7.0/80	0.40	Catalase 4	AAF61734.1	HA	(F)AYSDTQR(H) (K)YPINPAR(V) (L)GPNYLQLPVNAPK(C) (N)FPVFF(V) (N)FPVFFTR(D) (N)YLQLPVNAPK(C) (R)DEEVDYFPSR(Y) (R)EGNFDIVGNNFPVFFTR(D) (R)FSTVIHER(G) (R)LGPNYLQLPVNAPK(C) (S)SVNTLTLVNK(E) (Y)TLVNK(A)	APGV(Q/K)TPT(I/L)VR S(Q/K)AD(Q/K)S(I/L)G(Q/K)K	cd00328
				Stress and defense				
17	5.1/51	0.09	beta-1,3-glucanase	CAA09765.1	CI×CE		YVAVGNEVDPNK (I/L)YDPD(Q/K)NT(I/L)(Q/K)A(I/-L)(K	
			beta-1,3-glucanase	CAB55309.1	CI×CE		DN(I/L)(Q/K)NYPDVK	
18	5.0/51	0.03	beta-1,3-glucanase	BAA31142.1	CS	(R)TYNNNLIQHVK(Q)	—	
48	5.5/30	3.00	Putative chaperonin 21	AAT80888.1	VV	(V)SPGNTVLYSK(Y) (Y)TSLKPLGDR(V) (K)YTSLKPLGDR(V)	YAGNDF(Q/K)GPDGS(I/L)F(I/L)-DTR VGA(Q/K)VVYSK	cd00320
			Putative chaperonin 21	BAD35227.1	OSj	—	E(Q/K)PS(I/L)GTV(I/L)AVGPGP(I/-L)DDEG(Q/K)R	
			Triose phosphate isomerase	AAB23371.1	LS	(R)IIYGGSVSGSNC_mK(E) (R)EAGTTM_{ox}EVVAAQTK(A)		
				Miscellaneous				
5	4.9/29	2.50	Proteasome subunit alpha type	Q9M4T8	GM	(K)EGVVLAVEK(R) (R)TLVEHAR(V) (K)VTPNNVDIAK(V) (R)TEYDRGVNTFSPEGR(L) (K)QVM_{ox}EEKVTPNNVDIAK(V) (R)ITSPLLEPSSVEK(I) (R)FGEGDEESM_{ox}SRPF(G)	T(Q/K)DGVV(I/L)AVE(Q/K)R DGVV(I/L)AVE(Q/K)R VAPTYH(I/L)YTPAEVEAV(I/L)SRL DET(Q/K)F(Q/K)(Q/K)EYYN(Q/K-)A(I/L)C_mD(I/L)PSR	cd03753
12	6.6/19	2.50	Transcription factor	CAA70323.1	NPI	(R)LQSTLKR(L) (K)TTTTDDKRLQSTLKR(I) (Q)ANTWVVSGSPQTK(N)	(Q/K)(I/L)GPDN(I/L)DN(I/L)R	

Table 1: Continued.

Spot	pI /M_r (kDa)	Relative Fold change Green/Etiolated	Description	Accession number	Hortologous Organisms (c)	Exact Match sequence	"*de novo*" sequence	Conserved domains
				Carbon\Nitrogen metabolism or energy				
24	5.4/12	4.30	Glycine rich RNA binding protein	Q03878	DC	(I)NDRETGR(S) (I)INDRETGR(S) (R)EGGGGGYGGGGGYGGR(R) (R)C_mFVGGLAWATNDE(S)	AAADVEYR (I/L)DGRN(I/L)TVNEA(Q/K)SR	
				Unidentified proteins				
3	5.3/23	0.40	Unidentified proteins				(I/L)APYFCR TPPN(I/L)DPPR PYVR YMPAVFPEVF(Q/K)ADK PEVTK PFP(I/L)(I/L)V TPS(Q/K)YF(Q/K)T(Q/K)MDYPPP-GGTK T(I/L)DAGGSWY(I/L)VT(I/L)VEN	
4		0.20	Unidentified proteins				PVP(Q/K)K SEFT(Q/K)K	
6	4.6/30	2.50	Unidentified proteins				AANNETGR GEFSPYGESYPCR PVP(Q/K)K	
8	4.4/45	13.00	Unidentified proteins				MSCVSACGANFCK S(I/L)NYGAAMER CGPCNSDFR	
	(b) Constantly expressed proteins							
15	5.8/72	2.00	Mitochondrial processing peptidase	CAA56520.1	ST	(R)IVLAASGVEHEELLK(V)	DV(I/L)S(I/L)PTYDVVN EVEA(I/L)GGNVTASASR	
35	6.5/29	0.50	Triosephosphate isomerase cytosolic	P48493	LS	(K)VASPAQAQEVHAGLRK(W) (K)VASPAQAQEVHAGLR(K) (L)AYEPVWAIGTGK(V) (K)ISSWDNVVLAYEPVWAIG-TGK(V) (R)ALLNETNEFVGDKVAYAL-SQGLK(V) (K)AIADKISSWDNVVLAYEP-VWAIGTGK(V)		cd00311
39	5.9/29	0.60	Triosephosphate isomerase cytosolic	P48493	LS	(K)VASPAQAQEVH(A) (A)YEPVWAIGTGK(V) (R)IIYGGSVSGSNC$_m$K(E) (K)VIAC$_m$VGETLEQR(E) (R)ALLNETNEFVGDK(V) (R)EAGTTM$_{ox}$EVVAAQTK(A) (V)AAQTK(A)	M_{ox}YEVHAG(I/L)R	cd00311

Table 1: Continued.

Spot	pI /M_r (kDa)	Relative Fold change Green/Etiolated	Description	Accession number	Hortologous Organisms (c)	Exact Match sequence	"*de novo*" sequence	Conserved domains
Carbon\Nitrogen metabolism or energy								
			Triosephosphate isomerase cytosolic	P21820	OSj	(F)VGGNWK(C)	$PC_mGTSEDV(Q/K)K$	
40	5.4/19	0.80	ATP synthase D chain	Q9FT52	AT	(K)SLKESERLEK(E) (K)FDALLVELK(E) (L)LVELK(E) (I)ADVAFK(A) $(K)LSTM_{ox}TADEYFEK(H)$	FS(Q/K)EPEP(I/L)DWEYYRK VTDEA(Q/K)R (Q/K)VADVAFK	
41	5.3/20	1.90	ATP synthase D chain	Q9FT52	AT	$(R)TIDWDGM_{ox}AK(V)$ (L)LVELKEAEQK(S) (I)ADVAFK(A) $(K)LSTM_{ox}TADEYFEK(H)$ $(K)M_{ox}LVSDEAR(K)$ $(K)M_{ox}LVSDEARK(E)$	SVE(I/L)PK FS(Q/K)EPEP(I/L)DWEYYRK FS(Q/K)EPEP(I/L)DWEYYR	
49	6.6/51	1.00	Malate dehydrogenase	Q7XDC8	OS	(K)NVSIYK(A) (R)ALGQISEK(L) $(A)SSAC_mDHIR(-)$	F(I/L)STV(Q/K)(Q/K)R	
			Malate dehydrogenase	AAP70009.1	TA	(R)LGVQVSDVK(N)		
			Malate dehydrogenase	BAA97412.1	AT	(Q)ASALEK(H)		
			Malate dehydrogenase	AAF69802.1	VV	(K)TFYAGK(A)		
50	6.6/51	0.90	Malate dehydrogenase	AAF69802.1	VV	(L)FGVTTLDVVR(A) (T)FYAGK(A) (R)LFGVTTLDVVR(A) (R)TQDGGTEVVEAK(A) (T)EVVEAK(A) (K)TFYAGK(A) (S)SLSLYDIAGTPGVAADVSHIN-TR(S) $(K)VAVLGAAGGIGQPLA-LLM_{ox}K(L)$	R(I/L)FGVDS(I/L)DVVR RT(Q/K)DGGTEVVEAK A(I/L)EGADVV(I/L)(I/L)PAGVPR $SMC_mTA(I/L)AK$ (I/L)FGVTT(I/L)DVVR $AGA(I/L)FADAC_m(I/L)K$ N(I/L)SNEE(I/L)VA(I/L)TK NGVEEV(I/L)G(I/L)GS(I/L)SDYEK	cd01337
53	6.9/52	0.80	Malate dehydrogenase	AAC19244.1	GM	(R)LFGVTTLDVVR(A) (R)DDLFNINAGIVK(T) (K)ALEGADVVIIPAGVPR(K)		cd01337

Table 1: Continued.

Spot	pI /M_r (kDa)	Relative Fold change Green/Etiolated	Description	Accession number	Hortologous Organisms (c)	Exact Match sequence	"*de novo*" sequence	Conserved domains
			Carbon\Nitrogen metabolism or energy					
			Malate dehydrogenase	BAA97412.1	AT	(K)ASALEK(G) (K)SQASALEK(H) (K)NVSIYK(A)	F(I/L)STV(Q/K)(Q/K)R	
			Malate dehydrogenase	Q7XDC8	OS	(R)ALGQISEK(L)		
			Malate dehydrogenase	AAP70009.1	TA	(R)LGVQVSDVK(N)		
54	7.0/53	0.50	Malate dehydrogenase	AAF69802.1	VV	(R)TQDGGTEVVEAK(A) (R)DDLFNINAGIVK(T) (K)TFYAGK(A) (K)RTQDGGTEVVEAK(A) (K)SDYEK(M) (R)FYAGK(V) (C)TAIAK(Y)	VSS(I/L)S(I/L)YD(I/L)AGTPGVAAD-VSHMNAPA NGVEEV(I/L)G(I/L)GS(I/L)SDYEK	cd01337
			Malate dehydrogenase	AAD56659.1	GM	(N)VPVAGVNVPVVGGH(A) (K)ALEGADVVIIPAGVPR(K) (N)VPVAGV(N)	SEVVGY(Q/K)GDEEMGK	
56	5.5/74	0.50	ATPase beta subunit	AAD03393.1	NS	(R)IINVIGEPIDER(G) (R)EAPAFVEQATEQQILVTGIK(-V) (R)DAEGQDVLLFIDNIFR(F) (I)GLFGGAGVGK(T) (G)SPITVPVGR(A) (N)VIGEPIDER(G) (Y)GQM_{ox}NEPPGAR(A) (R)TIAM_{ox}DGTEGLVR(G) (R)VLNTGSPITVPVGR(A) (K)TVLIM_{ox}ELINNVAK(A) (R)FTQANSEVSALLGR(I) (K)C_mALVYGQM_{ox}NEPPGAR(A) (R)IPSAVGYQPTLATDLGGL-QER(I) (L)GEDHYNTAR(G)	(Q/K)NVA(Q/K)H(I/L)GYM_{ox}MR	cd01133
			Ribulose bisphosphate carboxylase RuBisCO large subunit	Q05987	CJ	(R)MSGGDHLHSGTVVGK(L) (L)GLSAK(N)		

Table 1: Continued.

Spot	pI /M_r (kDa)	Relative Fold change Green/Etiolated	Description	Accession number	Hortologous Organisms (c)	Exact Match sequence	"*de novo*" sequence	Conserved domains
				Carbon\Nitrogen metabolism or energy				
				Oxidative stress				
34	6.2/30	1.90	Glutathione S transferase PM24	P46422	AT	(G)EHKKEPFLSR(N)	A(I/L)T(Q/K)Y(I/L)AHRYEG(Q/K)G-TN(I/L)(I/L)PADSK	cd03053
				Stress and defence				
33	6.9/20	0.70	Pathogenesis-related protein 5-1	AAM21199.1	HA	(R)NNC$_m$PYTVWAGAVPGGGR(-Q)	AVFT(I/L)RNNCPYTVWAGAVPG-GGR TGC$_m$NFDGSGR	pfam00314
42	5.0/16	0.70	Pathogenesis-related protein	BAC10911.1	ZH	(K)VFSDFDNIAPK(V) (L)FTFGDAVPFTSGK(C) (K)QTIVYNCK(G) (K)LFTFGDAVPFTSGK(C)		
43	4.8/16	1.70	Pathogenesis-related protein	BAC10911.1	ZH	(K)VFSDFDNIAPK(V) (K)QTIVYNCK(G)	—	
59	5.1/100	1.50	Stromal 70 kDa heat shock related protein	Q02028	PS	(R)IPAVQELVK(K) (T)TLPTSK(S) (K)NQADSVVYQTEK(Q) (K)GPEGDVIDADFTDSK(-) (R)QAVVNPENTFFSVK(R) (R)IINEPTAA(S) (R)IINEPTAASLAYGFER(K) (V)PAYFNDSQR(T)	NTT(I/L)PTSK M$_{ox}$VSEAE(Q/K)FAK (Q/K)VSYTVVRDDNNNV(Q/K)(I/L-)ECPA(I/L)GK (I/L)PAV(Q/K)EVVK	COG0443
51	6.6/42	1.00	Phenylcoumaran benzylic ether reductase	CAA06706.1	PT	(K)ILIIGGTGYIGK(F) (I)LGDGNAK(A) (F)IVEASAK(S) (I)ILGDGNAK(A) (V)VEASAK(S)	(I/L)VA(I/L)K DVSDPSK D(Q/K)VV(I/L)(I/L)GDGNAK SFNE(I/L)VS(I/L)WEK (I/L)PT(I/L)A(Q/K)PAADGPPR	
52	6.7/42	0.50	Phenylcoumaran benzylic ether reductase homolog TH2	AAF64177.1	TH	(K)VVILGDGNAK(A) (L)GDGNAK(A) (R)DKVVILGDGNAK(A) (I)LGDGNAK(A) (V)ILGDGNAK(A)	(I/L)VA(I/L)K FGNDVDR YTTVDEY(I/L)TR	
				Miscellaneous				
26	5.7/13	1.10	Glycine rich RNA binding protein	Q03878	DC	(I)NDRETGR(S) (I)INDRETGR(S) (K)IINDRETGR(S)	DV(I/L)DS(Q/K)(I/L)(I/L)TDR DA(I/L)EGMSGEE(I/L)DGR TAFA(Q/K)YGDV(I/L)DSK GFGFVTF(Q/K)DE(Q/K)SMR N(I/L)TVNEA(Q/K)SR C$_m$FVGG(I/L)AWATGDAE	pfam00076

Table 1: Continued.

Spot	pI /M_r (kDa)	Relative Fold change Green/Etiolated	Description	Accession number	Hortologous Organisms (c)	Exact Match sequence	"*de novo*" sequence	Conserved domains
			Carbon\Nitrogen metabolism or energy					
			Glycine rich RNA binding protein GRP2A	P49311	SA	(M)ASPDVEYR(C)		
			RNA binding glycine rich protein 1 RGP 1a	BAA03743.1	NS	(G)SGGGGGGGGFRGGRREGG-G(G)	T(I/L)GEAFS(Q/K)FGE(I/L)(I/L)DSK	
27	5.9/17	0.70	Protein At5g10860 mitochondrial precursor	Q9LEV3	AT	(R)AM$_{ox}$QLM$_{ox}$TDNR(I)	H(I/L)R(Q/K)(I/L)(I/L)V(Q/K)GR VGD(I/L)M$_{ox}$TEEN(Q/K)(I/L)(I/L)-TVTPDTK TVTPDTK	
			Regulator of ribonuclease like protein 1	Q9M8R9	AT	(R)VLVVDGGGSLR(C)	VFEDNV(I/L)(I/L)R	
			Eukaryotic translation initiation factor 5A 1 eIF 5A 1	Q9XI91	AT	(R)ALQPVFQIYGR(R) (K)VVEVSTSK(T) (K)TYPQQAGTIR(K)		
37	5.8/30	1.40	Proteasome subunit alpha type 2A subunit B	O23708	AT	(K)ASNGVVLATEK(K) (T)AVGSGQTSLGIK(A) (K)EPIPVTQLVR(E) (D)DKGPQLYQVDPSGSYFSW-K(A) (R)YTEDMELDDAIHTAILTLK(E)	EGFEG(Q/K)(I/L)SGK PAAPS(I/L)(I/L)VDETEG(Q/K)K (I/L)YEEP(I/L)PVT(Q/K)(I/L)VR AC$_m$SNC$_m$SESMTTFSPSGK	cd03750
			Triosephosphate isomerase cytosolic	P48493	LS	(R)IIYGGSVSGSNC$_m$K(E) (R)EAGTTM$_{ox}$EVVAAQTK(A)		
57	5.4/68	1.30	26S protease regulatory subunit 6A homolog	O04019	AT	(K)LAGPQLVQM$_{ox}$FIGDGAK(L) (Q)M$_{ox}$FIGDGAK(L) (K)GVLLYGPPGTGK(T)		COG1222
44	5.0/41	1.40	atranbp1b (Ran-binding protein)	CAA66046.1	AT	(K)LYRFDK(D) (R)FGSVENC$_m$K(A)		
			Unidentified proteins					
58	5.0/64	0.70	Unidentified proteins				APDK DDPGR	

10 kDa protein that binds with high affinity to C_{12}–C_{22} acyl-CoA esters. *In vitro* and *in vivo* experiments suggest that it is involved in multiple cellular tasks including modulation of fatty acid biosynthesis, enzyme regulation, regulation of the intracellular acyl-CoA pool size, donation of acyl-CoA esters for β-oxidation, vesicular trafficking, complex lipid synthesis, and gene regulation. In higher plants, *de novo* fatty acid biosynthesis occurs in the chloroplasts. Plastid-synthesized fatty acyl-CoAs, palmitoyl-CoA (16 : 0-CoA), and oleoyl-CoA (18 : 1-CoA) are likely exported by intracellular lipid transporters to the endoplasmic reticulum (ER) for the "eukaryotic pathway" in lipid biosynthesis. The process as to how the fatty acyl-CoAs are exported from the chloroplasts to the ER remains unclear to date. The ACBP that was first reported in *Arabidopsis* was proposed to facilitate transport and maintenance of long-chain acyl-CoA esters in the cytosol. This assumption was based on a lack of knowledge at that time that five other ACBPs exist in *Arabidopsis* [28]. Recently studies suggest that those proteins are light-regulated [29]; in fact we observed an increased value of this protein in the etiolated flesh stalks.

Moreover, some enzymes involved in electron transport and energy production are downregulated. When exposed to dark, photosynthetically competent chloroplast developed into photosynthetically incompetent etioplast. This involved the destruction of component of the photosynthetic apparatus and prononouced alterations in plastid ultrastrutture that include the conversion of stromal and stacked thylakoid structures characteristic of chloroplast into distinctive prolamellar body. In this study, vacuolar ATP synthase (spot 25), the photosystem II oxygen-evolving complex protein 1 (spots 45, 46, 47), and photosystem II oxygen-evolving complex protein 2 (spot 36) were downregulated (Table 1). Infact, the oxygen evolving protein plays an important role in stabilizing, binding, and maintaining functional conformation of the manganese cluster which directly catalyzes the H_2O-splitting reaction [30]. After exposure to the dark the protein decreases significantly, indicating that the oxygen evolving complex of PSII was flourished by exposure to light.

3.3. Proteins Associated with Oxidative Stresses. Etiolation has been described as an oxidative process which might lead to the production or accumulation of a large amount of harmful active oxygen species (AOS) or, more specifically, of hydrogen peroxide as a by-product of increased electron transport activity in the mitochondria. The presence of damaging hydrogen peroxide might explain the strong presence of ascorbate peroxidase, with the function of contrasting toxic peroxide [31].

L-ascorbate peroxidase activity (spot 38) showed a remarkable decreasing value in etiolated as compared to green fleshy stalks. Ascorbate is a major metabolite in plants. It is an antioxidant and, in association with other components of the antioxidant system, protects plants against oxidative damage resulting from aerobic metabolism, photosynthesis, and a range of pollutants. In fact, the degradation of the photosynthetic apparatus reduces the presence of the L-ascorbate peroxidase.

Catalase (cd00328) (spot 55) was identified as a homologue of *Heliantus annuus* catalase 4 and was also upregulated in etiolated fleshy stalks. The increase in antioxidant enzymes was indicative of a pro-oxidant signal under a nonphotosynthetic condition. Besides, glutathione S-transferase (spot 34) was similar in green and etiolated fleshy stalks.

3.4. Proteins Associated with Stress and Defense. Proteins homologous to stromal 70 kDa heat-shock protein (spot 59) were detected in both green and etiolated fleshy stalks. The chaperonin 21 (spot 48) was downregulated; it has been demonstrated that this protein has a more general role in plastid development and may influence the folding of numerous chloroplast protein such RuBisCO (EC 4.1.1.39) [32].

The beta 1,3 glucanase (spots 17,18) proteins are upregulated and are involved in the response to biotic stresses that are differentially expressed during etiolation.

Besides, phenylcoumaran benzylic ether reductase (PCBER) (spots 51, 52) was identified as protein constantly expressed, which plays a significant role in the biosynthesis of important plant defense phenylpropanoid-derived compounds, based on known catalytic function component presiding over defense mechanisms. These proteins possess NADPH binding sites and appear to be cytosolic enzymes, since they lack secretory pathway signal sequences. In addition, they may be regulated in a comparable manner, not only at the transcriptional level but also at the enzymatic level by protein phosphorylation, as evidenced by several potential phosphorylation sites that are conserved among all of the members [33].

Enzymatic assay with recombinant Bet v 6 and Pyr c 5 showed PCBER catalytic activity for both recombinant allergens [34]. Therefore, PCBER may represent a new class of cross-reactive allergens of birch pollen and plant food.

3.5. Miscellaneous. Information on several identified proteins that did not group in the above classes was very poor; in particular, their function was postulated on the basis of the homology sequence criteria. In only a few cases, important functions in some physiological processes have emerged for definite proteins. In this respect, it is worth noting that three proteins are involved in the control of programmed cell death (PCD), namely, the proteasome alpha subunit type 2A (spot 37), 26S protease regulatory subunit 6A (spot 57), and proteasome alpha subunit type 5 (spot 5) containing a ubiquitin-like domain interacting with catalytically active proteasomes [35]. Increasing evidence indicates that PCD plays a pivotal role in diverse plant processes, such as embryogenesis, senescence, or pathogen defense [36, 37], and we find out that the proteasome alpha subunit type 5 (spot 5) is downregulated and decreased its value in the etiolating process but data are lacking on its involvement in this process.

Ran-binding protein 1b, homologous to that from *Arabidopsis* (AtRanBP1b), was also identified (spot 44). Ran-binding proteins are activators of Ras-related nuclear small GTP-binding proteins (Ran) and in *Arabidopsis* have been

Table 2: Protein expressed exclusively in etiolated cultivated cardoon. Spot number, experimental *pI* and M_r values, protein description, accession number, hortologous organism, peptide sequences, and conserved domain are listed. (a) Abbreviated names of organisms: AT: *Arabidopsis thaliana*; GM: *Glycine max*; SS: *Solenostemon scutellarioides.*

					Miscellaneous		
Spot	pI/M_r (kDa)	Description	Accession number	Hortologous Organism (a)	Exact Match Sequence	"*de novo*" Sequence	Conserved domains
29	6.1/19	CBS domain-containing protein	ABK64186.1	SS	(K)LITVTPDTK(V)	AVVSEHREE(I/L)DR	
					(R)AMQLMTDNR(I)		
					(K)VGDIMTEENKLITVTPDTK(V)		
		Eukaryotic translation initiation factor 5A	Q9XI91	AT	(K)VVEVSTSK(T)		
					(K)TYPQQAGTIR(K)		
32	6.9/20	Protein P21	P25096	GM	(R)VVFCP(-)		
					(R)TGCNFDGSGR(G)		

shown to be involved in the regulation of auxin-induced mitotic progression [38]. Another identified protein for which information is very scarce or missing was the glycine-rich RNA-binding protein (spot 24) which decreases its value in the etiolation process. Besides, two proteins are exclusively expressed during etiolation (spots 29 and 32; Table 2) in the fleshy stalks.

Spot 29 is a cystathionine beta-synthase (CBS), domain-containing protein also present in the proteome of archaeobacteria, prokaryotes, and eukaryotes, having divergent functions ranging from metabolic enzymes and transcriptional regulators to ion channels and transporters. Although the precise function has not yet been elucidated, CBS domains could have physiological importance because point mutations are responsible for several hereditary diseases in humans [39].

Protein P21 (P25096) (spot 32) is classified as a ras-oncogene-encoded p21 protein in humans and is known to cause large numbers of tumours. The protein differs from its healthy counterpart in eukaryotic cells in that it contains single amino acid substitutions at critical positions of the polypeptide chain, such as Gly^{12}, Gly^{13}, Ala^{59}, and Gln^{61} [40]. In a recent study [41], one quantitative trait locus (QTL), fw2.2, has been demonstrated as being responsible for the domestication of the tomato. When transformed into large-fruited cultivars, a cosmid derived from the fw2.2 region of a small-fruited wild species reduced fruit size by the predicted amount and had the gene action expected for fw2.2. The cause of the QTL effect is a single gene, ORFX, expressed early in the floral development. It controls carpel cell number and its sequence has structural features similar to that of human oncogene c-H-ras p21

4. Concluding Remarks

The 634,985 proteins of the *Viridiplantae* database, including the complete genome of *Arabidopsis thaliana* and other important plants, have allowed identification of protein expression profiles of *Cynara cardunculus* under usual light or dark conditions.

In this study, constitutive and differentially expressed proteins in green and etiolated fleshy stalks of cultivated cardoon (*Cynara cardunculus* L. var. *altilis* DC) have been discussed in relation to the plant etiolation process. In fact, the proteomic study indicated that, during etiolation, the differentially expressed proteins were involved mainly in starch metabolism and stress response. The major group in response to dark exposure was found to be proteins related to PSII complex. Thus groups comprised four proteins (spots 36; 45; 46; 47) encoded by two nuclear genes (PSBO; PSBP). The decreasing values of these proteins explain the role that the PSII complex plays in the early photosynthetic phase. In fact its downregulation during the etiolating process is correlated to a progressive loose of function such as the oxidation of water to molecular oxygen. Therefore, the rapid decrease in the relative amount of these proteins following dark exposure suggests that the PSII complex is the light-driven water: plastoquinone oxidoreductase.

In conclusion, this study allowed to identify the proteins that are implicated in different physiological and defence process besides their involvement in the response to stress, as the etiolation process. This one is to be considered as a reaction that helps plants to survive in the dark condition, contributing to the response at the physiological effects due to this exposure.

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