Jozef Šamaj • Jay J. Thelen (Editors)

Plant Proteomics

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With 32 Figures, 7 in color and 13 Tables

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Jozef Šamaj received his Ph.D. degree in Plant Physiology from the Comenius University in Bratislava, Slovakia. He completed three post-doctoral programmes supported by Eurosilva, the Alexander von Humboldt Foundation, and the EU Marie Curie Programme in the highly regarded laboratories of Alain Boudet in Toulouse, Dieter Volkmann in Bonn, and Heribert Hirt in Vienna. He worked on the cell biology of somatic embryogenesis, lignification in tree species, arabinogalactan proteins, the cytoskeleton, and signalling proteins. Jozef Šamaj has co-edited three books and co-authored more than 75 research papers, reviews, and book chapters. He is a senior lecturer and group leader at the Institute of Cellular and Molecular Botany in Bonn, Germany, and senior researcher at the Institute of Plant Genetics and Biotechnology, Slovak Academy of Sciences, Nitra, Slovakia. His current research is focussed on the role of signalling components and the cytoskeleton in relation to the vesicular trafficking during plant development and stress responses using integrated cell-biological and functional proteomics approaches.

Jay Thelen received his B.Sc. degree in Biology and Biochemistry from the University of Nebraska-Lincoln in 1993. He earned his Ph.D. from the University of Missouri-Columbia (UMC) studying the structure and regulation of plant mitochondrial pyruvate dehydrogenase complexes under the guidance of Douglas Randall. In 1999 he started a 3-year postdoctoral position in John Ohlrogge's lab at Michigan State University investigating the plastid acetyl-CoA carboxylase protein complex. He returned to UMC in 2002 as the Associate Director of a campus Proteomics Center. In 2004, he was promoted to Assistant Professor in the Biochemistry Department, a position he currently holds. He has authored or co-authored 35 research and review articles since 1994. His research interests are centered around the regulation of plant metabolism, particularly carbon assimilation in oilseeds, and multienzyme metabolic complexes. He is currently studying seed filling in numerous crop oilseeds, using various quantitative proteomics approaches. He is also investigating global phosphoprotein networks involved in seed development and is developing improved strategies for quantitative proteomics.

Contents

Preface

Plant proteomics is a relatively new research field focused on the large-scale functional analysis of proteins extracted from intact plants, particular plant organs, tissues, individual cells, subcellular organelles and/or separated suborganellar structures. Rapidly increasing numbers of excellent publications on plant proteomics, both in primary and applied research, demonstrate the imense potential and importance of this research field for current and future plant science. One of the main aims of plant proteomics is to study the assembly and functional interactions of plant proteins. Proteins often function as molecular machines organized into multiprotein complexes localized within specialized subcellular compartments. Enormous methodological and technical developments have moved recent proteomics towards the large-scale study of post-translational modifications of proteins involved in cellular signalling (regulated by reversible phosphorylation), protein turnover (ubiquitinylation) and membrane association (palmitoylation and myristylation).

This book highlights this rapid progress in plant proteomics with emphasis on model species, subcellular organelles as well as specific aspects such as signalling, plant reproduction, stress biology and/or pathogen/symbiotic interactions between plants and microorganisms. Additionally, brief historical overviews on plant proteomics and two-dimensional gel electrophoresis as well as an introduction to bioinfomatics are provided here. Thus, this monograph represents a synthesis of the most current knowledge in this field, including the most important biological aspects as well as new methodological approaches such as high-resolution two-dimensional electrophoresis, protein microchips, MudPIT (multidimensional protein identification technology), fluorescent DIGE (difference gel electrophoresis) alone and/or in combination with stable isotope reagents such as ICAT (isotope-coded affinity tag) and iTRAQ (isobaric tag for relative and absolute quantitation), which allow relative protein quantification. The reader is provided with an up-to date view on plant proteomes in carefully selected model plant species such as Arabidopsis, cereals, legumes and oil seed plants. One chapter focuses on the cell division model represented by suspension cultured tobacco BY-2 cells. Several chapters are devoted to the proteomics of plant organelles and compartments. Among the latter, special attention is paid to the cell wall, plasma membrane, plastids, mitochondria and nucleolus. Two chapters focus on proteomic approaches used to study plant reproduction, namely pollen proteomics and the proteomics of

seed development in oilseed crops. Finally, four chapters describe proteomes during pathogenic and symbiotic interactions between plants and microrganisms, and during plant stress responses. Regarding future perspectives, it is very important that diverse integrated approaches including advanced proteomic techniques combined with functional genomics, bioinformatics, metabolomics and/or with advanced molecular cell biology are nicely presented in several chapters. Thus, this book not only covers the rapid progress in the field of plant proteomics but also delivers this recent knowledge to a broad spectrum of readers including advanced students, teachers and researchers.

At this point I would like to thank my co-editor Jay Thelen and all the authors for their great job and excellent contributions to this book. Last but not least, my special thanks goes to my family, my wife Olinka and sons Matejko and Tomáško, for their encouragement and patience with me during this book project.

Bonn, April 2007 Jozef Šamaj

List of Abbreviations

Chapter 1

PAGE	polyacrylamide gel electrophoresis
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
LC	liquid chromatography
2-D	two-dimensional
IPG	immobilized pH gradient
IEF	isoelectric focusing
CBB	Coomassie Brilliant Blue
ESI	Electrospray Ionization
MALDI	Matrix Assisted Laser Desorption Ionization
TOF	Time of Flight
PMF	peptide mass fingerprinting
EST	expressed sequence tag
ICAT	Isotope-Coded Affinity Tag
MS	Mass spectrometry

Chapter 2

2-DE	Two-dimensional gel electrophoresis
MS	mass spectrometry
IPG	immobilized pH gradients
NEPHGE	non-equilibrium pH gradient electrophoresis
mABC1	mitochondrial ATP-binding cassette protein 1
SB 3-10	N-decyl-N, N-dimethyl-3-ammonio-1-propane sulfonate
TBP	tributyl phosphine
DIGE	difference gel electrophoresis
MALDI	Matrix-assisted laser desorption/ionization
TOF	Time of flight
CID	collision-induced
IEF	isoelectric focusing
SDS-PAGE	sodium dodecyl sulfate polyacrylamide

	gel electrophoresis
EST	expressed sequence tag
CBB	Coomassie Brilliant Blue

Chapter 3

CID	collision-induced dissociation
PTM	post-translational modification
GPM	global proteome machine

Chapter 4

MAPK	mitogen-activated protein kinase
MAPKKKs	MAPK kinase kinases
MAPKKs	MAPK kinases
PP2C	Protein Ser/Thr phosphatase 2C
DsPs	Ser/Thr/Tyr phosphatases
PTP	protein Tyr phosphatase
MS	mass spectrometry
LC-MS	liquid chromatography-mass spectrometry
IMAC	immobilised metal ion affinity chromatography
SCX	strong cationic exchange
SRPK	SR protein-specific kinase
SILAC	Stable Isotope Labelling by Amino acids in Cell culture
TiO_2	Titanium dioxide
EST	expressed sequence tag

Chapter 5

2-DE	two-dimensional gel electrophoresis
MS	mass-spectrometry
UPA	universal protein array
PMAs	protein microarrays
AMAs	antibody microarrays
RPMAs	reverse protein microarrays
HMG	high mobility group
ACS-6	1-aminocyclopropane-1-carboxylic acid synthase-6
MKS1	MAPK substrate 1
VSP1	vegetative storage protein1
Pro-Q DPS	ProQ-Diamond phosphoprotein strain
CK2α	casein kinase2α
MPK	mitogen-activated kinase
PKA	Protein kinase A

Chapter 6

ECM	extracellular matrix
ER	endoplasmic reticulum

GAPDH	glyceraldehyde-3-phosphate dehydrogenase
2D-DiGE	2-dimensional difference gel electrophoresis
2-DE	2-dimensional gel electrophoresis
MS	mass-spectrometry
GFP	green fluorescent protein
FB1	fumonisin B1

Chapter 7

2-DE	2-dimensional gel electrophoresis
BN-PAGE	blue native-polyacrylamide gel electrophoresis
CK2α	casein kinase 2α
DIGE	difference gel electrophoresis
DLC	diamond-like carbon coated stainless steel
ESI	electrospray ionization
FT	Fourier transform ion cyclotron resonance
GFP	green fluorescent protein
ICAT	isotope-coded affinity tag
IMAC	immobilized metal affinity chromatography
IT	ion trap
iTRAQ	isobaric tag for relative and absolute quantitation
LC	liquid chromatography
MALDI	matrix-assisted laser desorption ionization
MS	mass spectrometry
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
PTM	post-translational modification
Q	quadrupole
TAP	tandem affinity purification
TOF	time-of-flight
2-DE	two-dimensional electrophoresis

Chapter 8

MS	mass-spectrometry
MALDI	matrix-assisted laser desorption ionization
Q	quadrupole
TOF	time-of-flight
ESI	electrospray ionization
eSLDB	eukaryotic sub-cellular localisation database
SUBA	subcellular location database for Arabidopsis proteins
GFP	green fluorescent protein
AMPDB	Arabidopsis Mitochondrial Protein Database
IMAC	metal ion affinity chromatography
TRX	thioredoxin
TAP	tandem affinity purification

TEV	tobacco etch virus
ICAT	isotope-coded affinity tag
iTRAQ	isobaric tag for relative and absolute quantitation
SILAC	stable isotope labeling with amino acids in cell culture
MRM	multiple reaction monitoring
HPLC	high performance liquid chromatography
LC	liquid chromatography

Chapter 9

2-DE	two-dimensional electrophoresis
Mb	Megabase
EST	expressed sequence tag
TC	tentative consensus
ppm	parts per million
IEF	isoelectric focusing
AM	arbuscular mycorrhizal
dai	days after inoculation
PR	pathogenesis-related
ABA	abscisic acid
Ado-Met	S-adenosyl-Met
LEA	late embryogenesis abundant
A. euteiches	Aphanomyces euteiches
NSF	N-ethylmaleimide-sensitive fusion
ABA	abscisic acid
G. mosseae	Glomus mosseae
G. intraradices	Glomus intraradices
iTRAQ	isobaric tags for relative and absolute quantitation
ICAT	isotope-coded affinity tags
IPG	immobilized pH gradient
LC-MS/MS	liquid chromatography coupled to tandem mass spectrometry
MALDI TOF-MS	matrix assisted laser desorption/ionization time-of-flight mass spectrometry
MS	mass spectrometry
MS/MS	tandem mass spectrometry
M. truncatula	Medicago truncatula
MudPIT	multidimensional protein identification technology
pI	isoelectric point
PMF	peptide mass fingerprinting
PR	pathogenesis-related
RuBisCO	ribulose 1,5-bisphosphate carboxylase/oxygenase
S. meliloti	Sinorhizobium meliloti.

Chapter 10

WAF	weeks after flowering

2-DE	two-dimensional gel electrophoresis
PTM	post-translational modifications
PMF	peptide mass fingerprint
TC	tentative consensus
IPG	immobilized pH gradient
MALDI-TOF	Matrix Assisted Laser Desorption Ionization-Time of Flight
EST	expressed sequence tag
SSP	seed storage proteins
LOX	lipoxygenases
SuSy	sucrose synthase
SBP	sucrose-binding protein
PEP	phosphoenolpyruvate
PDC	pyruvate dehydrogenase complex
FBA	fructose bisphosphate aldolase
RuBisCO	ribulose-1,5-bisphosphate carboxylase/oxygenase
	3-PGA 3-phosphoglycerate
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
PGK	phosphoglycerate kinase
PGM	phosphoglucomutase
PGI	phosphoglucose isomerase
TPI	triose-phosphate isomerases
iPGAM	2,3-bisphosphoglycerate-independent phopsphoglycerate mutase
PK	pyruvate kinase
LEA	late embryogenesis
IEF	isoelectric focusing
TAG	triacylglycerol
ER	endoplasmic reticulum
BiP	luminal binding protein
MS/MS	tandem mass spectrometry
CBB	Coomassie Brilliant Blue

Chapter 11

EST	expressed sequence tag
BY-2	Bright Yellow-2
2-DE	two dimensional gel-electrophoresis
PTM	post-translational modification
MS	mass spectroscopy
PMF	peptide mass fingerprint
DiGE	difference in gel electrophoresis
iTRAQ	isobaric tags for relative and absolute quantification
LC	liquid chromatography
IPG	immobilised pH gradient
SDS-PAGE	sodium dodecyl sulfate polyacrylamide

	gel electrophoresis
IEF	isoelectric focusing
LC-ESI-Q-TOF MS	liquid chromatography–electrospray ionisation–quadrupole–time-of-flight mass spectrometry
MALDI	matrix-assisted laser desorption/ionization
MS/MS	tandem MS
LPS	lipopolysaccharides
RuBP	ruthenium II tris bathophenanthroline disulfonate
ROS	reactive oxygen species
BN	blue native
RuBisCO	ribulose 1,5-bisphosphate carboxylase/oxygenase

Chapter 12

CWP	cell wall protein
HRGPs	Hyp-rich glycoproteins
H/PRPs	Hyp/Pro-rich proteins
GRPs	Gly-rich proteins
GH	glycoside hydrolases
XTH	xyloglucan endotransglucosylase/hydrolases
PMEs	pectin methylesterases
LRX	leucine-rich repeat-extensins
AGPs	arabinogalactan proteins
MS	mass spectrometry
PTM	post-translational modifications
CWME	cell wall modifying enzymes
CWMEI	inhibitors of cell wall modifying enzymes
PG	polygalaturonase
XEGIPs	xyloglucan endoglucanase inhibiting proteins
GPI	glycosylphosphatidylinositol
MALDI-TOF	Matrix-assisted laser desorption/ionization-time of flight
TDIF	tracheary differentiation inhibitory factor
PGIPs	inhibitors of polygalacturonases
PMEI	Inhibitor of PME
Nt-CIF	inhibitor of tobacco invertase
ER	endoplasmic reticulum

Chapter 13

MS	mass spectroscopy
RLKs	receptor-like protein kinases
IEF	iso-electrofocusing
CTAB	cationic trimethyl ammonium bromide
BN-PAGE	blue-native electrophoresis
MuDPIT	multidimensional protein identification technique

MALDI	matrix assisted laser desorption/ionisation
ESI	electrospray ionisation
PI-PLC	phosphatidylinositol phospholipase C
IMAC	immobilised metal ion affinity chromatography
LDS	lithium dodecyl sulphate
LOPIT	localisation of organelle proteins by isotope tagging
GPI	glycosylphosphatidylinositol
iTRAQ	isobaric tags for relative and absolute quantification
ER	endoplasmic reticulum
2-DE	two-dimensional gel electrophoresis
16-BAC	benzyldimethyl-n-hexadecylammonium chloride
CNBr	cyanogen bromide
GO	gene ontology
DIGE	difference in gel electrophoresis
ESTs	expressed sequence tags

Chapter 14

MS	mass spectroscopy
PTM	post-translational modification
TIC	Translocon at the Inner envelope membrane of Chloroplasts
TOC	Translocon at the Outer envelope membrane of Chloroplasts
2-DE	two-dimensional gel electrophoresis
TAT	twin-arginine translocation
TPR	tetratricopeptide
PPR	pentatricopeptide
PAP	plastid lipid-associated protein
ER	endoplasmic reticulum
ceQORH	chloroplast envelope quinone oxidoreductase homologue
IEP32	inner envelope protein of 32 kDa
CAH1	carbonic anhydrase 1
Clp	caseinolytic protease
IEF	isoelectric focusing

Chapter 15

BSA	bovine serum albumin
PVP	polyvinylpyrrolidone
FW	fresh weight
PAGE	Polyacrylamide gel electrophoresis
IEF	isoelectric focusing
SDS-PAGE	sodium dodecyl sulfate PAGE

MS	mass spectrometry
BN	blue native
TOM	translocase of the outer membrane
HSPs	heat shock proteins
CMS	cytoplasmic male sterility
PTM	post-translational modification
PDC	pyruvate dehydrogenase complex
TCA cycle	tricarboxylic acid cycle
AOS	active oxygen species
ROS	reactive oxygen species
HNE	4-hydroxy-2-nonenal
TRX	thioredoxin
Y2H	yeast two-hybrid technique
FRET	fluorescence resonance energy transfer
BRET	bioluminescence resonance energy transfer
GRAVY	grand average of hydrophobicity

Chapter 16

NoLS	Nucleolar localisation sequences
PLRV	potato leaf-roll virus
GFP	green fluorescent protein
2-DE	2D polyacrylamide gel electrophoresis
LC	liquid chromatography
MS	mass spectrometry
Mr	relative molecular mass
DiGE	difference gel electrophoresis
MuDPIT	multidimensional protein identification technique
MALDI	matrix assisted laser desorption/ionisation
ESI	electrospray ionisation
MS/MS	tandem MS
TOF	time-of-flight
FT-ICR-MS	Fourier-transform ion-cyclotron resonance mass spectrometer
snRNP	small nuclear RNP
PTMs	Post-translational modifications
GPI	glycosylphosphatidylinositol
SILAC	Stable Isotope Labelling by Amino acids in Cell culture
ICAT	isotope coded affinity tagging
LOPIT	Localisation of organelle proteins by isotope tagging
ER	endoplasmic reticulum
ITRAQ	Isobaric tag for relative and absolute quantification
TAP	tandem affinity purification
TEV	tobacco etch-virus
CBP	calmodulin-binding protein

EF	elongation factor
EST	expressed sequence tag

Chapter 17

PTMs	Post-translational modifications
MS	mass spectrometry
MS/MS	tandem mass spectrometry
2-DE	two-dimensional gel electrophoresis
GO	gene ontology
EST	expressed sequence tag
Q	quadrupole
ESI	electrospray ionisation
MALDI-TOF	matrix assisted laser desorption/ionisation–time-of-flight

Chapter 18

PAMPs	pathogen-associated molecular patterns
PRs	pathogenesis-related proteins
SAR	systemic acquired resistance
ROS	reactive oxygen species
PR	pathogenesis-related
SA	salicylic acid
JA	jasmonic acid
ET	ethylene
MS	mass spectrometry
AM	arbuscular mycorrhizal
2D-LC	two dimensional liquid chromatography
MS/MS	tandem mass spectrometry
Nep1	necrosis- and ethylene-inducing peptide
HR	hypersensitive response
MAPKs	mitogen-activated protein kinases
EST	expressed sequence tag
CSI	cross-species identification
AVR	avirulence
R	resistance
RLP	receptor-like protein
CITRX	Cf-9-interacting thioredoxin
NBS-LRR	nucleotide binding site leucine-rich repeat
GIP	glucanase inhibitor proteins
PI	protease inhibitors
TGases	transglutaminases
CBEL	cellulose binding elicitor lectin
PAMP	pathogen-associated molecular pattern
NLPs	Nep1-like proteins
CBD	cellulose-binding domain

PRLs	PR-like proteins
PAL	phenylalanine ammonia-lyase
TMV	tobacco mosaic virus
FHB	Fusarium head blight
SOD	superoxide dismutase
HRGP	hydroxyproline-rich glycoprotein
SAR	systemic acquired resistance

Chapter 19

2-DE	two-dimensional electrophoresis
MALDI	matrix assisted laser desorption ionisation
PMF	peptide mass fingerprinting
CDPK	calmodulin-like domain protein kinase
NDPK	Nucleoside diphosphate kinase

Chapter 20

AM	arbuscular mycorrhizal
1D-SDS PAGE	one dimensional sodium dodecyl sulfate polycacrylamide gel
2-DE	two-dimensional gel electrophoresis
LC	liquid chromatography
ESI-MS/MS	electrospray ionisation tandem mass spectrometry
ESI-Q-TOF	electrospray ionisation quadrupole time of flight
ESTs	expressed sequence tags
GPI	glycosylphosphatidylinositol
HPLC	high performance liquid chromatography
IPG	immobilized pH gradient
MALDI-TOF	matrix assisted laser desorption ionisation time of flight
PCR	polymerase chain reaction
PM	plasma membrane
RNAi	RNA interference
TILLING	targeting induced local lesions in genomes
PMF	peptide mass fingerprint
MS/MS	tandem mass spectrometry
HPLC	high performance liquid chromatography
DIGE	2-D difference gel electrophoresis
ICAT	isotope coded affinity tag
MUDPIT	multidimensional protein identification technology
RNAi	RNA interference

Chapter 21

MS	Mass spectrometry
MALDI-TOF	matrix assisted laser desorption ionisation time of flight

ABA	Abscisic acid
RuBisCO	ribulose 1,5-bisphosphate carboxylase/oxygenase
HSP	heat shock protein
2-DE	two-dimensional electrophoresis
PR	pathogenesis-related
ASR	ABA/stress/ripening responsive protein
COMT	caffeate-O-methyltransferase
SAM	S-adenosyl-L-methionine
QTLs	quantitative trait loci
PQL	protein quantity locus
LEA	late embryogenesis abundant
CV	coefficient of variation
ROS	reactive oxygen species
DIGE	Difference in-gel electrophoresis
PMF	peptide mass fingerprint
EIFs	eukaryotic initiation factors
SOD	superoxide dismutase
PG	plastoglobule
PC	phytochelatin
GSH	glutathione
BN	Blue native
GST	Glutathione S-transferase

Chapter 1
Introduction to Proteomics: a Brief Historical Perspective on Contemporary Approaches

Jay J. Thelen

Abstract The field of proteomics has experienced numerous milestones over the course of the past 35–40 years. As an introductory chapter to this larger review text on plant proteomics, this article provides a cursory historical perspective on protein separation and identification techniques widely used in plant biochemistry laboratories today. In the past 10 years alone, advancements in techniques such as two-dimensional gel electrophoresis, mass spectrometry, and mass spectral data mining have made previously intractable proteomics problems almost routine by today's standards. In analyzing these various proteomics approaches I also discuss and project their utility for the next generation of proteomics research.

1.1 Introduction

Proteomics, or the high-throughput identification and analysis of proteins, is an emerging field of research facilitated by numerous advancements over the past 35–40 years in protein separation, mass spectrometry, genome sequencing/annotation, and protein search algorithms. Recognizing this trend in the physical and life sciences, the term "proteome" was first used by Wilkins et al. (1995) to describe the protein complement to the genome. Since the first use of this term its meaning and scope have narrowed. The host of post-translational modifications, alternative splice products, and proteins intractable to conventional separation techniques has each presented a challenge towards the achievement of the classic definition of the word (Chapman 2000; Westermeier and Naven 2002; Wilkins and Gooley 1998). The broad dynamic range of protein expression has also contributed to difficulties in efforts towards identifying every protein expressed in the life cycle of any given organism (Corthals et al. 2000). For example, identification of every protein expressed in plant leaves would never reveal proteins that are specifically expressed

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in roots. Despite these limitations, hundreds if not thousands of proteins can be resolved, profiled and identified using the latest methods – a remarkable achievement given the recent genesis of this discipline.

The purpose of this chapter is to briefly introduce and provide a historical perspective on established proteomics concepts and methods that are being used in many plant biology laboratories today to comparatively profile protein expression and identify proteins. I will also attempt to provide a perspective on the future outlook of each of these approaches. This introduction will hopefully be useful for non-experts in the field of proteomics as an aid to comprehension of most of the terminology and jargon used in this highly technical field of life sciences research. The varied approaches to proteomics research can be generally classified as having one of two major objectives: (1) protein or peptide separation, and (2) identification and characterization of resolved proteins or peptides, typically by mass spectrometry. I will address these two aspects of proteomics research in the first two sections in this introductory chapter and then discuss general strategies for quantitative protein profiling.

1.2 Protein Separation and Detection for Proteome Investigations

Currently, there are three preferred methods for separation of complex protein or peptide samples: (1) denaturing polyacrylamide gel electrophoresis (PAGE) also referred to as sodium dodecyl sulfate polyacrylamide (SDS-PAGE); (2) two-dimensional (2-D) gel electrophoresis; and (3) liquid chromatography (LC) a general term that includes all forms of ion exchange, affinity, and reversed-phase chromatography (Hunter et al. 2002). There are of course other forms of protein separation, including preparative isoelectric focusing (protein separation according to native charge) and native or blue-native PAGE, to name but a few alternative techniques. Due to space constraints however, only SDS-PAGE and 2-D gel electrophoresis will be discussed here.

1.2.1 Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis

No protein separation technique is more widely used than SDS-PAGE, first reported by Laemmli in 1970. It would not be an exaggeration to state that nearly all contemporary laboratories performing life sciences research employ this technique. The widespread use of SDS-PAGE to separate proteins according to size can be attributed to its ease, reproducibility, and modest consumable and instrument expenses. Although an easy technique to perform, the resolving power of SDS-PAGE is somewhat limited. Mass spectrometry (MS) analysis of any single discreet SDS-PAGE protein band from a complex protein sample consistently reveals multiple proteins, frequently greater than ten (Phinney and Thelen 2005). However, for highly enriched

samples of low complexity (<10 unique proteins) SDS-PAGE may be suitable. In general, accurate quantitative analysis of SDS-PAGE protein bands from a complex sample is not feasible as the volume of any band is the collective composition of each unique protein in that band. However, as a pre-fractionation technique for alternative quantification strategies including chemical labeling (using stable isotope conjugates; Ramus et al. 2006) and perhaps label-free quantification using recently developed software tools (SIEVE, DeCyder MS), SDS-PAGE may find a new niche as a rapid, reproducible separation technique prior to MS quantification.

1.2.2 Two-Dimensional Gel Electrophoresis

Around the time at which SDS-PAGE was introduced, O'Farrell applied isoelectric focusing (IEF) to protein samples prior to SDS-PAGE to pioneer the concept of two-dimensional (2-D) gel electrophoresis (O'Farrell 1975). Although extremely powerful in its resolving capabilities, this method suffered from reproducibility issues owing to the casting, focusing, and extrusion of the fragile tube gels used for IEF. Over the years this procedure has been improved through the introduction in 1978 (Görg et al. 1978) and recent commercialization (Görg et al. 2000) of the immobilized pH gradient (IPG) strip, to replace IEF tube gels, which has resulted in a major resurgence in this technique.

Reproducibility, sample loading and resolution for 2-D gel electrophoresis have significantly improved with the introduction of the IPG strip in conjunction with commercial Peltier-cooled programmable focusing units for IEF (Görg et al. 2000). These advancements have made 2-D electrophoresis an attractive method for the separation of complex protein samples. Besides the impressive separation capabilities, another reason 2-DE is frequently preferred to LC-based approaches for protein separation is that a reproducible 2-DE proteome reference map is a static, visual entity. A fully annotated 2-DE reference map for a specific organ, tissue, cell, or organelle of interest is a valuable tool that can save time and money when 'landmarking' differentially expressed proteins in response to a treatment, mutation, or transgene introduction. Although 2-D electrophoresis suffers from well-publicized limitations, such as under-representation of membrane proteins (Wilkins et al. 1998; Santoni et al. 2000), this time-honored method is presently one of the preferred approaches for quantitative characterization of complex protein samples. The popularity of 2-DE will no doubt continue with recent technical developments such as sensitive and quantitative pre- and post-electrophoretic stains for total proteins, as well as phospho- and glycoproteins, as discussed herein.

1.2.3 Extracting Proteins From Plant Samples

Performing 2-D electrophoresis with plant samples can be a challenging endeavor, in part due to the high carbohydrate:protein ratio in most plant tissues. Direct grinding of samples in IEF extraction media, while generally sufficient for non-plant