Modulation of Proteome and Phosphoproteome Under Abiotic Stress in Plants: An Overview

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Abbreviations

2D-PAGE	Two-dimensional polyacrylamide gel electrophoresis
DIGE	Differential in gel electrophoresis
ESI	Electrospray ionization
ICAT	Isotope-coded affinity tags
IMAC	Immobilized metal affinity chromatography
iTRAQ	Isobaric tags for relative and absolute quantification
LC	Liquid chromatography
LCM	Laser capture micro-dissection
MALDI-TOF	Matrix assisted laser desorption/ionization-time of flight
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MudPit	Multidimensional Protein Identification Technology
PTM	Post-translational modification
SILAC	Stable isotope labelling by amino acids in cell cultures

1 Introduction and Brief Bibliographic Review

Plant growth, development and productivity are severely diminished by abiotic stress factors such as drought, salinity, waterlogging, extreme temperatures and heavy metals (Surabhi 2018). As a consequence to it, physiological and biochemical responses in plants vary and cellular aqueous and ionic equilibriums are disrupted

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(Sreenivasulu et al. 2007). The assessment of potential yield losses by individual abiotic stresses is estimated at 17% (drought), 20% (salinity), 40% (high temperature), 15% (low temperature) and 8% by other factors (Shafiq-ur-Rehman and Ashraf 2005). It has been estimated that 90% of arable land experience different abiotic stresses, singly or in combination (Leopold 1990) under field conditions. Plant responses to abiotic stresses are dynamic and complex, and quite different depending on the type, level, duration of the stress involved, type of tissue and genotype under stress (Cramer et al. 2011). Higher plants have evolved multiple, interconnected strategies that enable them to survive under abiotic stress (Surabhi et al. 2003; Kumari et al. 2007; Surabhi et al. 2008; Veeranagamallaiah et al. 2008; Singh et al. 2010; Witzel et al. 2009, 2010; Surabhi 2018). However, these strategies are not well developed in most agricultural crops (Fig. 1).

Unlike genome which is a static structure inherited from parents and defining plant genotype, changes in plant epigenome, transcriptome, proteome and metabolome shape plant phenotype in response to both developmental stages and for external cues. Plant stress proteomics is a dynamic discipline, aimed at studying plant proteome and protein biological functions in plants exposed to various stress factors (Veeranagamallaiah et al. 2008; Witzel et al. 2009, 2010; Surabhi 2018). The role of proteins in plant stress response is crucial since proteins are directly involved in shaping novel phenotype by adjustment of physiological traits to altered environment.

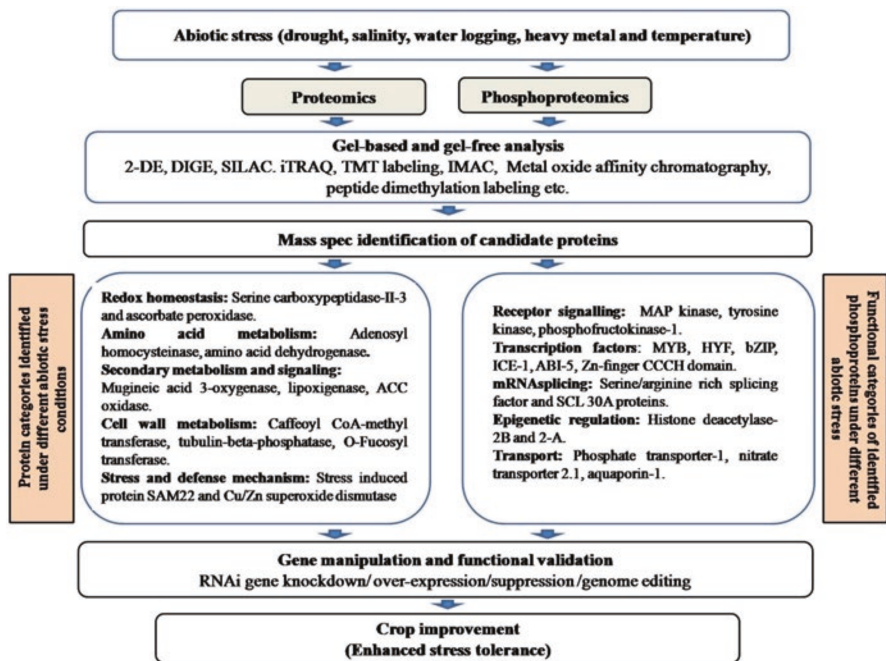

Fig. 1 Schematic representation of the proteomic strategy to study proteome and phosphoproteome modulations under different abiotic stresses in crop plants

Further, the analysis of protein is the most direct approach to define the function of its associated gene, proteome analysis linked to genome-sequence information is a very powerful tool in functional genomics studies (Komatsu et al. 2003). There are several types of proteomes that can be measured under abiotic stress, and each of them can reveal particular information about the expressed proteins. The most common proteomes to be measured in crop/plant or abiotic stress related studies are the whole proteome and the phosphoproteome (Helmy et al. 2012a, b; Witzel et al. 2009, 2010; Surabhi 2018). Phosphorylation is one of the most important post-translational modifications (PTMs) of proteins (Pawson and Scott 1997). Approximately one-third of the proteins are modified by phosphorylation (Hubbard and Cohen 1993). The study of whole proteome and phosphoproteome are the quantitative and/or qualitative profiling of all the expressed proteins and phosphorylated proteins in a given sample, respectively (Nakagami et al. 2012). Through phosphoproteomics, proteins and signalling pathways involved in response to particular stress can be identified (Sugiyama et al. 2008; Lassowskat et al. 2014; Zhang et al. 2014). Both proteome and phosphoproteome can be combined in one study to obtain a holistic understanding of abiotic stress tolerance in plants (Margaria et al. 2013; Yang et al. 2012; Hopff et al. 2013).

In recent years, proteome and phosphoproteome studies were conducted in crop/plants singly or in combination of both (Chitteti and Peng 2007; Margaria et al. 2013; Yang et al. 2012; Hopff et al. 2013) to get an molecular insight under different abiotic stresses such as drought (Atikur et al. 2016; Paul et al. 2015; Simova-Stoilova et al. 2015a, b; Li et al. 2018; Zadraznik et al. 2017; Hu et al. 2015; Bhaskara et al. 2017; Ren et al. 2017), salinity (Mostek et al. 2015; Zhang et al. 2017, 2018; Pi et al. 2018; Witzel et al. 2009), temperature (Guo et al. 2017; Gao et al. 2017; Pi et al. 2017), waterlogging (Pan et al. 2010, 2018; Mustafa and Komatsu 2014) and heavy metal stress (Xue et al. 2015; Chen et al. 2015, Cheng et al. 2017; Zhong et al. 2017). This review highlights some of the recent proteomics and phosphoproteomics studies conducted on crop/plants under different abiotic stresses. In addition, this review briefly discussed about different proteins which were altered in crop/plants under different abiotic stress factors. Finally, functional studies should complement high-throughput proteome analysis and can thus contribute to uncover protein role in plant stress response (Table 1).

2 Summary of Proteome and Phosphoproteome Studies Under Different Abiotic Stresses

Plant stress proteomics has the ability of identifying possible candidate genes that can be used for the genetic enhancement of plants to different stress factors (Cushman and Bohnert 2000; Rodziewicz et al. 2014; Barkla et al. 2016). Proteomics deals with determination, identification, expression profiling, post-translational modifications (PTMs) and protein–protein interactions under stress conditions (Hashiguchi et al. 2010; Nam et al. 2012; Mertins et al. 2013; Ghosh and Xu 2014). Using a proteome

Table 1 Major protein functional categories and proteins identified under different abiotic stress conditions in crop plants

Proteomics		Phosphoproteomics	
Functional categories of proteins	Name of the proteins	Functional categories of proteins	Name of the proteins
Drought stress			
Secondary metabolism (Simova-Stoilova et al. 2015a, b). Protein synthesis (Faghani et al. 2015; Simova-Stoilova et al. 2015a, b; Li et al. 2015). Carbon metabolism (Simova-Stoilova et al. 2015a, b). Glycolysis (Simova-Stoilova et al. 2015a, b; Atikur et al. 2016). Amino acid metabolism (Simova-Stoilova et al. 2015a, b). Adenosine triphosphate (ATP)-synthesis (Faghani et al. 2015; Simova-Stoilova et al. 2015a, b). Protein folding (Simova-Stoilova et al. 2015a, b; Zadraznik et al. 2017). Protein degradation (Simova-Stoilova et al. 2015a, b). Stress response (Paul et al. 2015; Simova-Stoilova et al. 2015a, b; Zadraznik et al. 2017). Photosynthesis (Faghani et al. 2015; Simova-Stoilova et al. 2015a, b). Signal transduction (Li et al. 2015; Atikur et al. 2016). Oxidative stress (Faghani et al. 2015; Atikur et al. 2016; Ghaffari et al. 2013). Energy metabolism (Ghaffari et al. 2013; Paul et al. 2015; Li et al. 2015; Atikur et al. 2016). Transport (Ghaffari et al. 2013; Li et al. 2015). Defense (Faghani et al. 2015, Li et al. 2015). Carbohydrate metabolism (Paul et al. 2015). Cell wall metabolism (Faghani et al. 2015; Zadraznik et al. 2017).	Caffeoyl-CoA-30 methyl transferase and chalcone synthase (Simova-Stoilova et al. 2015a, b). 30-S ribosomal protein S-7 and 40-S ribosomal protein S-17-4 (Li et al. 2015), Protein disulfideisomerase and 60S ribosomal protein L-13 (Faghani et al. 2015), DEAD-box RNA helicase and glycyl t-RNA synthetase (Simova-Stoilova et al. 2015a, b). Methyl malonate dehydrogenase, methylene tetrahydrofolatedreductase (Simova-Stoilova et al. 2015a, b). Glucose-6-phosphate isomerase and phosphofructokinase-1 (Simova-Stoilova et al. 2015a, b), oxaloacetate, malate dehydrogenase (Atikur et al. 2016). Glutamine synthase and cysteine synthase (Simova-Stoilova et al. 2015a, b). ATP-synthase gamma-chain and ATP-synthase mitochondrial (Faghani et al. 2015), ATP-synthase subunit-beta, NADH-unbiquinoneoxidoreductase (Simova-Stoilova et al. 2015a, b). Protein disulphide isomerise,chapperonin 16-Kda (Simova-Stoilova et al. 2015a, b) and UDP-glucose-glycoprotein glycosyltransferase, disulfide dismutase (Zadraznik et al. 2017). Amino peptidase and mitochondrial processing peptidase (Simova-Stoilova et al. 2015a, b).	Stress (Zhang et al. 2014; Hu et al. 2015). Defense and ROS (Zhang et al. 2014; Ke et al. 2009). Signal transduction (Zhang et al. 2014; Umezawa et al. 2013; Ke et al. 2009; Hu et al. 2015; Chen et al. 2017; Sun et al. 2017, Barua et al. 2019). Transport (Zhang et al. 2014; Sun et al. 2017; Yuan et al. 2016). Transcription factors (Zhang et al. 2014; Hu et al. 2015; Sun et al. 2017; Yuan et al. 2016). RNA processing (Zhang et al. 2014). Translational activator (Ren et al. 2017). Post-transcriptional regulation (Hu et al. 2015).	A-20 like-zinc finger and AN-1 zinc finger (Zhang et al. 2014) and Serine pyruvate dehydrogenase (Hu et al. 2015). Glutamate-decarboxylase-1 and phosphorylate glutathione peroxidise (Zhang et al. 2014), Germin like protein-1 (GLP-1) and putative r40-C-1 like protein (Ke et al. 2009). Snrk and Pb-2-C-kinase (Zhang et al. 2014), Phospholipase and phosphoenol pyruvate carboxykinase (Hu et al. 2015), CDPK-kinases and Snrk-2 kinases (Umezawa et al. 2013), Receptor like protein-kinase-1 and serine threonine protein kinase (Hu et al. 2015), CDK-Kinases and MAP-kinases NTF3 (Barua et al. 2019), G-beta subunit like protein kinases (Ke et al. 2009), protein receptor kinase (Chen et al. 2017), CDK-kinases (Sun et al. 2017). S-1-26 protein kinase-A and CDPK-1 kinase (Zhang et al. 2014), Aquaporin and SOS signalling (Sun et al. 2017), Aquaporin NIP-2 and cationic amino acid transporter (Yuan et al. 2016).

Cell growth and division (Li et al. 2015). Cell structure (Li et al. 2015). Transcription factors (Faghani et al. 2015; Li et al. 2015). Protein storage (Li et al. 2015). Metabolism (Li et al. 2015).	Endo-1,3-beta-glucanase and chitinase (Paul et al. 2015), Aldo/keto-reductase (Simova-Stoilova et al. 2015a, b), ascorbate oxidase and reticulin (Zadraznik et al. 2017). Oxygen evolving enhancers proteins ribulose-1,5-biphosphate carboxylase activase (Faghani et al. 2015) and Oxygen-evolving enhancers proteins and psbp domain-containing proteins (Simova-Stoilova et al. 2015a, b). *S*-adenosyl methionine synthase and calmodulin-3 (Li et al. 2015), Cytosolic—ascorbate peroxidase (Atikur et al. 2016). Ascorbate peroxidase hairpin binding protein-1 (Faghani et al. 2015), Peroxy reducing and APX-peroxidase (Atikur et al. 2016), Ascorbate peroxidase-2 and Cu/Zn-superoxide dismutase (Ghaffari et al. 2013). ATP-citrate-lyase and adenosine triphosphate citrate lyase (Ghaffari et al. 2013), Pyrophosphoglycerate adenosine-kinase (Paul et al. 2015), Early-induced protein 22 and glyceraldehydes 3-phosphate dehydrogenase (Li et al. 2015) and mitochondrial dihydrolipoyl dehydrogenase (Atikur et al. 2016). Aldo/keto-reductase and cysteine synthase (Atikur et al. 2016). HSP-21 (Ghaffari et al. 2013), Lipid transfer protein (Li et al. 2015). Pathogenesis related protein (Faghani et al. 2015), Thylakoid ascorbate peroxidase protein CuT A1 (Li et al. 2015). Phosphoglucomutase (Paul et al. 2015). Glyceraldehydes-3-phosphate dehydrogenase fructose bisphosphatealdolase (Faghani et al. 2015), Beta-xylosidase and alpha-aribinofuranosidase (Zadraznik et al. 2017). Tetratricopeptide repeat protein (Li et al. 2015). Cell structure (Li et al. 2015). 14-3-3-protein basic transcription factor 3 (Faghani et al. 2015), Cp31BHv (Li et al. 2015). Cucumisin and GrPE protein (Li et al. 2015). Inorganic phosphatase (Li et al. 2015).	mRNA splicing (Chen et al. 2017; Sun et al. 2017; Barua et al. 2019). Starch biosynthesis (Chen et al. 2017). Protein folding (Sun et al. 2017). Epigenetic regulation (Sun et al. 2017). Pyruvate metabolism (Sun et al. 2017). Water and ion transport (Sun et al. 2017).	MYB1R1 and bHLH (Zhang et al. 2014), 2-amino-ethanethiol dioxygenase like protein and HSP-COP 2 N6 (Hu et al. 2015), Transcriptional adapter ADA-2 and probable nucleolar protein 5-2 (Yuan et al. 2016), bZR1 and HUA1 (Sun et al. 2017), Zinc-finger CCCH domain and TF-PoS F-21 (Barua et al. 2019). CDPK-kinases and Snrk-2 kinases (Umezawa et al. 2013). GCN-1 and eukaryotic translational factor 3-subunit—B (Ke et al. 2009). Splicing factor 3-B-subunit-1 (Zhang et al. 2014), Serine/arginine rich-splicing factor-4 (Chen et al. 2017), serine arginine rich proteins, SCL 30A proteins (Sun et al. 2017) and E-3 Sumo-ligase SIZ 1 protein (Barua et al. 2019). Amlyopectin and amylases (Chen et al. 2017). Ribosomal protein S-6 and S6K-TOR protein (Sun et al. 2017). Histone deacetylase 2-B (Sun et al. 2017). Phosphoenolpyruvate carboxylase-4 and malate dehydrogenase (Sun et al. 2017). NIP-2 and PiP-27 phosphorylase (Yuan et al. 2016).

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Salt stress			
Amino acid metabolism (Mostek et al. 2015; Zhang et al. 2017, 2018). Ribosome activation (Zhang et al. 2017, 2018). Energy metabolism (Shen et al. 2016; Wen et al. 2016; Zhang et al. 2017, 2018). Protein folding (Gao et al. 2011, Mostek et al. 2015, Wen et al. 2016, Zhang et al. 2017, 2018). Nucleotide metabolism (Zhang et al. 2017, 2018). Cell division and cell signalling (Zhang et al. 2017, 2018). Membrane trafficking (Zhang et al. 2017, 2018). Glycolysis (Shen et al. 2016; Zhang et al. 2017, 2018). Citrate metabolism (Zhang et al. 2017, 2018). Pentose phosphate pathway (Zhang et al. 2017, 2018). Starch sucrose metabolism (Zhang et al. 2017, 2018). Defense (Witzel et al. 2014; Muneer and Jeong 2015; Wen et al. 2016). Primary metabolism (Witzel et al. 2014). Protein destination and storage (Witzel et al. 2014). Redox homeostasis (Witzel et al. 2014; Gao et al. 2011; Mostek et al. 2015; Shen et al. 2016). Secondary metabolism (Witzel et al. 2014). Carbohydrate metabolism (Gao et al. 2011; Mostek et al. 2015; Cui et al. 2015; Wen et al. 2016). Protein transport (Gao et al. 2011). Carbon metabolism (Gao et al. 2011). Signal transduction (Mostek et al. 2015). Cell wall metabolism (Mostek et al. 2015). RNA splicing (Muneer and Jeong 2015). Transcription regulation (Muneer and Jeong 2015). Starch biosynthesis (Muneer and Jeong 2015). RNA-binding (Muneer and Jeong 2015).	Serine hydroxyl methyl transferase and *S*-adenosyl L-homocysteine hydrolase (Mostek et al. 2015), Adenosine homo-cysteinase (Zhang et al. 2017, 2018). Phosphotransferase and phosphofructokinase-1 (Zhang et al. 2017, 2018). ATP-synthase subunit alpha and gamma (Shen et al. 2016), V-type proton ATPase catalytic subunit A and ATP synthase CF1 beta-subunit (Wen et al. 2016), Pyruvate orthophosphate dikinase (Zhang et al. 2017, 2018). Protein disulphide isomerases (Mostek et al. 2015; Zhang et al. 2017, 2018), CPN-60 alpha-protein (Wen et al. 2016). FAD-binding berberine family (Zhang et al. 2017, 2018). ATP-dependent CLP protease (Zhang et al. 2017, 2018). Coatamer alpha-subunit (Zhang et al. 2017, 2018). Phosphoglycerate kinase and triose-phosphate isomerase (Shen et al. 2016), Triose phosphate isomerase (Zhang et al. 2017, 2018). Pyruvate phosphate dikinase-2 (Zhang et al. 2017, 2018). UDP-glucose dehydrogenase (Zhang et al. 2017, 2018). Glycosyl hydrolases (Zhang et al. 2017, 2018). Germin like protein 12 and pathogenesis related protein 10 (Witzel et al. 2014), NBS/LRR-disease resistance proteins and PR-P10 proteins (Muneer and Jeong 2015), Betaine-aldehyde-dehydrogenase and glutamine synthetase cytosolic isoenzyme (Wen et al. 2016). Lipoxygenase-1 and platidiclipoxygenase-pyrophosphatase (Witzel et al. 2014). Proteasome subunit—alpha type-1 (Witzel et al. 2014). Glutathione transferases (Witzel et al. 2014), Chaperonnin HSP-mutase (Gao et al. 2011), Linolate 9*S*-lipoxygenase-1 and annexintranslationally controlled tumour lipoxygenase-2 (Mostek et al. 2015), Succinyl-CoA-ligase beta-chain and hydroxyacyl glutathione hydrolase (Shen et al. 2016). Mugineic acid 3-dioxygenase (Witzel et al. 2014).	ROS homeostasis (Jun et al. 2010; Pi et al. 2018; Yu et al. 2016). Signal transduction (Jun et al. 2010; Chang et al. 2012; Yu et al. 2016). Cellular regulation (Jun et al. 2010). Post-translational modification (Jun et al. 2010). Energy metabolism (Jun et al. 2010). Carbohydrate metabolism (Jun et al. 2010; Wen et al. 2016). Transport (Hsu et al. 2009; Wen et al. 2016; Chang et al. 2012; Yu et al. 2016). Water channel (Hsu et al. 2009). Protein folding (Hsu et al. 2009). Regulatory mechanism (Wen et al. 2016). Stress (Wen et al. 2016). Defense (Wen et al. 2016). Protein folding (Wen et al. 2016; Yu et al. 2016). Secondary metabolism (Pi et al. 2018). Transcription factors (Pi et al. 2018; Chang et al. 2012; Yu et al. 2016).	Cu/Zn-Superoxide dismutase-1 and glutamate decarboxylase (Jun et al. 2010), ascorbate peroxidase (Pi et al. 2018), glutathione-*s*-transferase and glutathione reductase (Yu et al. 2016). TPX-1 tyrosine kinase and phosphofructokinase-1 (Jun et al. 2010), receptor kinase and CDPK kinase (Chang et al. 2012), MAP-kinases and serine/threonine protein kinase (Yu et al. 2016). Zinc peroxide dismutase-1 (Jun et al. 2010).

Cellular metabolic process (Muneer and Jeong 2015). Nucleotide binding (Muneer and Jeong 2015). Stress (Muneer and Jeong 2015; Wen et al. 2016). ATP-binding (Muneer and Jeong 2015). Glycerol metabolic process (Muneer and Jeong 2015). Ribosome related protein (Cui et al. 2015). Regulatory mechanism (Wen et al. 2016). Protein metabolism (Wen et al. 2016). Photosynthesis (Wen et al. 2016). Cell structure (Wen et al. 2016).	Fructose 1,6-bisphosphatedolase and ribulose-1,6-bisphosphate aldolase (Gao et al. 2011), Citrate synthase and pyrophosphate fructose-6-phosphate phophotransferase (Mostek et al. 2015), Exhydrolase-2-isoform-I and fructokinase-2 (Cui et al. 2015). Putative H^+ transporting ATP-synthase (Gao et al. 2011). Chaperonnin HSP-mutase (Gao et al. 2011). Glutathione *S*-transferase (Gao et al. 2011). Carboxylase 3-phosphoglycerate kinase (Gao et al. 2011). UDP-glucoronic acid decarboxylase and beta-D-glucan-exohydrolase (Mostek et al. 2015). Osmotinvacuolar ATP-ase and thaumatin like protein (Mostek et al. 2015). Maturase (Muneer and Jeong 2015). Transcription elongation factor protein SPT-4 (Muneer and Jeong 2015). Granule bound starch synthase (Muneer and Jeong 2015). S-46 RNase (Muneer and Jeong 2015). Cinnamoyl-CoA-reductase like-1 Muneer and Jeong 2015). Pentatricopeptide repeat containing protein (Muneer and Jeong 2015). Zinc finger nucleases A-20 and AN-1 domain (Muneer and Jeong 2015) Chloroplast—Cu/Zn superoxide dismutase and dehydroascorbatereductase (Wen et al. 2016). 4-coumarate CoA-ligase (Muneer and Jeong 2015). Putative DAK-2-domain containing protein (Muneer and Jeong 2015). Ribosomal protein S-8 and 60S ribosomal protein L-3 (Cui et al. 2015). CP31-BHv (Wen et al. 2016). 50S ribosomal protein L-1 and 50S ribosomal protein L10 (Wen et al. 2016). Fructose-biphosphatealdolase and soluble inorganic pyrophosphatase-1 (Wen et al. 2016). Oxygen evolving enhancer protein-2 (Wen et al. 2016). Ribulose-biphosphate carboxylase and oxygenase (Wen et al. 2016). Enolyl-(acyl carrier protein)-reductase NADH and actin (Wen et al. 2016).		NADH-dehydrogenase-1 (Jun et al. 2010). NADH-dehydrogenase-2 (Jun et al. 2010). Fructose bisphosphate aldolase and NADH dehydrogenase (Jun et al. 2010), Triosphosphate-isomerase and chloroplast fructose bisphosphatealdolase (Wen et al. 2016), sugar transport protein 1 and aquaporin PIP-2 (Chang et al. 2012), PIP-2-1 and NHX-1 protein (Yu et al. 2016). Purine permease (Hsu et al. 2009), ATP synthase CF1 beta subunit and ATP1 synthase (Wen et al. 2016). Aquaporin-PIP-2 (Hsu et al. 2009). FAM-10 protein translase (Hsu et al. 2009). Cp31BHv and Cp31BHv (Wen et al. 2016). Cu/Zn superoxide dismutase and dihydroascorbate (Wen et al. 2016). Chain-A beta glucosidase and 2-cysteine peroredoxin BAS1 (Wen et al. 2016). CPN-60 alpha protein (Wen et al. 2016), 60s ribosomal protein L17 and TRAP (translocon associated protein sub-unit) (Yu et al. 2016). P-Coumanocyl-CoA and alonyl-CoA (Pi et al. 2018). PsbH1 and PsbH2 (Chang et al. 2012), bHLH and SYF1 (Yu et al. 2016), MYBIR and MYB-R2R3 (Pi et al. 2018).

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Waterlogging			
Carbohydrate metabolism (Alam et al. 2010; Yin and Komatsu 2017). Energy metabolism (Alam et al. 2010; Yin and Komatsu 2017). Signal transduction (Alam et al. 2010). Programmed cell death (Alam et al. 2010). Redox homeostasis process NAD (Alam et al. 2010; Yin and Komatsu 2017; Oskuei et al. 2017; Kamal et al. 2015; Wang et al. 2015; You et al. 2014; Li et al. 2018). Nitrogen metabolism (Alam et al. 2010). DNA processing (Alam et al. 2010). Post-translational modification (Alam et al. 2010). Cell wall metabolism (Li et al. 2018; Yin and Komatsu 2017; Wang et al. 2015; Khan and Komatsu 2016). RNA metabolism (Li et al. 2018). Protein Transport (Li et al. 2018; Khan and Komatsu 2016; Yin and Komatsu 2017). DNA metabolism (Li et al. 2018). Lipid metabolism (Kamal et al. 2015; Wang et al. 2015; Li et al. 2018). Protein metabolism (Li et al. 2018). Glycolysis (Yin and Komatsu 2017). Defense (Yin and Komatsu 2017). Protein synthesis (Yin and Komatsu 2017). Transcription factor (Yin and Komatsu 2017; You et al. 2014). Glycosylation (Yin and Komatsu 2017). Protein modification (Yin and Komatsu 2017). Biotinylation (Yin and Komatsu 2017). Calcium homeostasis (Yin and Komatsu 2017). Photosynthesis (Oskuei et al. 2017). Cell wall modification (Xue et al. 2015). Phenyl propanoid metabolism (Xue et al. 2015). Lipid catabolism (Xue et al. 2015).	UDP-glucose pyrophosphorylase and carbohydrate-kinases (Alam et al. 2010), *S*-adenosyl methionine synthetase and B-*S*-glucosidase 44 (Yin and Komatsu 2017). Phosphofructokinase and auxin-amidohydrolase (Alam et al. 2010), Enolase and alcohol dehydrogenase (Yin and Komatsu 2017). Receptor kinase and ERK kinase (Alam et al. 2010). Sam synthase and apyrase (Alam et al. 2010). NADH-hydrolase and ascorbate peroxidase (Alam et al. 2010), Ascorbate peroxidase and N-ascorbate dehydrogenase (Yin and Komatsu 2017), Ascorbate peroxidase mono-dehydrate ascorbatereductase (Oskuei et al. 2017), Fructose bisphosphate aldolase-2 (Kamal et al. 2015), PD1 like 16 protein (Wang et al. 2015). Glutamine synthase (Alam et al. 2010). Glucose-6-phosphate/phosphate translocator related protein (Li et al. 2018), mitochondria substrate family carrier protein (Yin and Komatsu 2017), Nucleotidyltransferase and granulin repeat cysteine protease (Khan and Komatsu 2016), Potassium phosphatase beta subunit-A (Wang et al. 2015). AATF-leucine zipper domain protein (Alam et al. 2010). Deoxyhypusine synthase (Alam et al. 2010). O-fucosyl-transferase family protein (Li et al. 2018), Xylosidase (Yin and Komatsu 2017). DNA (Cytosin-5) methyl transferase and RNA recognition motif (Li et al. 2018), Reversibly glycosylated polypeptide 3 (Wang et al. 2015). Oxidoreductase and pyridoxal-5-phosphate dependent enzyme (Wang et al. 2015), Xyloglucanendotransglycosylase (You et al. 2014). Replication factor C2 (Li et al. 2018), Ribosomal LSP-family protein (Kamal et al. 2015). Putative uncharacterized protein (Li et al. 2018).	Energy metabolism (Pan et al. 2018). Sucrose metabolism (Pan et al. 2018). Pentose phosphate pathway (Pan et al. 2018). Transcription factor (Pan et al. 2018). Carbohydrate metabolism (Cho et al. 2016). Transport (Yin et al. 2014; Cho et al. 2016). Signal transduction (Yin et al. 2014; Cho et al. 2016). Protein synthesis (Yin et al. 2014; Cho et al. 2016). Cell wall metabolism (Yin et al. 2014). Nucleotide metabolism (Yin et al. 2014). RNA processing (Yin et al. 2014). Hormone metabolism (Yin et al. 2014). Glycolysis (Yin et al. 2014).	Pyruvate kinase and phosphoenol pyruvate carboxylase (Pan et al. 2018). Glucose-6-phosphate dehydrogenase (Pan et al. 2018). Ribulose-1,5-biphosphate carboxylase (Pan et al. 2018). Serine protein kinase and threonine protein kinase (Pan et al. 2018). F2KP and plastidic acetyl—CoA synthetase (Cho et al. 2016). Proton antiporter and PIP-2 (Cho et al. 2016), transmembrane amino acid transporter family protein (Yin et al. 2014). SNF-kinase-1 (Yin et al. 2014), SnRK1 and MPK kinase-3 (Cho et al. 2016).

Secondary metabolism (Khan and Komatsu 2016). Glycolysis (Khan and Komatsu 2016). Protein transport (Khan and Komatsu 2016). Stress (Khan and Komatsu 2016; Kamal et al. 2015; Wang et al. 2015; Li et al. 2018). Protein synthesis (Kamal et al. 2015). Hormone metabolism (Kamal et al. 2015; Wang et al. 2015). Photosynthesis (Kamal et al. 2015). Nucleotide metabolism (Kamal et al. 2015). Protein degradation (Kamal et al. 2015). Protein activation (Kamal et al. 2015). Nucleotide metabolism (Wang et al. 2015). Cell signalling (Wang et al. 2015; Kamal et al. 2015; Li et al. 2018). Amino acid metabolism (Wang et al. 2015). RNA processing (Wang et al. 2015). Secondary metabolism (Wang et al. 2015). TCA cycle (Wang et al. 2015). Glycolysis (Wang et al. 2015; You et al. 2014). Protein transport (Wang et al. 2015). Protein metabolism (You et al. 2014). Sucrose metabolism (You et al. 2014). Glycolysis (You et al. 2014). Lactate fermentation (You et al. 2014). Ethylene biosynthesis (You et al. 2014). Defense (You et al. 2014). Secondary metabolism (You et al. 2014).	Stress induced protein SAM22 (Li et al. 2018), MLP-like protein (Khan and Komatsu 2016), leucine rich repeats protein (Kamal et al. 2015), HSPs 70 and DNA damaged binding protein (Wang et al. 2015). RAS-GTP transcription activator (Yin and Komatsu 2017), Glutamine synthase (You et al. 2014). Proteasome subunit alpha type and NAG-CoA transferase (Li et al. 2018). Stress induced proteins and SAM-22 (Li et al. 2018). Tubulin beta1 chain (Kamal et al. 2015; RAS-5 and RAS-GTP-binding protein (Wang et al. 2015). Ferritin proteins (Li et al. 2018). ATP-citrate lyase (Yin and Komatsu 2017). UDP-glucose-6-hydrogenase (Yin and Komatsu 2017), Plant intracellular RAS group-LRR-4 and calmodulin binding transcription activator CG-1 (Li et al. 2018), Phospholipase-D and oxophytodienoatereductase (Xin et al. 2016). Eukaryotic translation initiation factor 4-G (Yin and Komatsu 2017). Zinc finger/BTB-domain protein 47 (Yin and Komatsu 2017). Calnexin/calreticulin beta-xylosyltransferase (Yin and Komatsu 2017). Foldase and dnaJ protein (Yin and Komatsu 2017). Class-II-aminoacyltRNA-biotin synthetase (Yin and Komatsu 2017). Calcium transportinATPases (Yin and Komatsu 2017). Carbonic anhydrase-2 and aldolone-type TIM barrier family (Oskuei et al. 2017). Xyloglucanendo-transglucosylase/hydrolases and polygalacturonases (Xue et al. 2015). PAL proteins (Xue et al. 2015). Lipoxygenases and esterases/lipases (Xue et al. 2015). Chalcone flavanone isomerase and beta keto acyl reductase (Khan and Komatsu 2016). MLP-like protein (Khan and Komatsu 2016). Pyruvate protein (Khan and Komatsu 2016).		Translation elongation factor EF1B/ribosomal protein S6 and glutathione-*s*-transferase c-terminal (Yin et al. 2014), 60s acidic ribosomal protein family and ribosomal protein L10 family proteins (Cho et al. 2016). UDP-glucose 6-dehydrogenase (Yin et al. 2014). AMP deaminase (Yin et al. 2014). TUDOR-SN1 protein (Yin et al. 2014). Aluminium induced protein with YGL and LRDR (Yin et al. 2014). Phosphoglucomutase (Yin et al. 2014).

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Table 1 (continued)

	Leucine rich repeat LRR (Kamal et al. 2015). Plant stearoylacyldesaturase carrier protein (Kamal et al. 2015). Ascorbate peroxidase 1 (Kamal et al. 2015), Uridine diphosphate glycosyl transferase (Wang et al. 2015). Pyridine 2 (Kamal et al. 2015). Tubulin-beta-1 chain (Kamal et al. 2015). Regulatory particle triple A1A-protein and 20S proteasome alpha subunit E2 (Kamal et al. 2015). Class-II aminoacyltRNA and biotin synthetases (Kamal et al. 2015). Lipoyl domain protein and ATP-dependent caseinolytic protease (Wang et al. 2015). Pyrophosphorylase (Wang et al. 2015). RAS-5, RAS-GTP binding protein (Wang et al. 2015). RNA binding protein CTC interacting domain II (Wang et al. 2015). GHMP kinase (Wang et al. 2015), Phosphofructokinase and triosphosphateisomerase (You et al. 2014). Dihydrolipoamide acetyl transferase (Wang et al. 2015). Phosphoglucomutase and phosphomannomutase (Wang et al. 2015). Glutathione synthase (You et al. 2014). Glutathione-*S*-transferase and glutathione dehydrogenase (You et al. 2014). Alanine amino-transferase and aminotransferase 3 (You et al. 2014). Sucrose synthase-4 and phosphoglucomutase (You et al. 2014). Lactate dehydrogenase (You et al. 2014). ACC oxidases (You et al. 2014). Thioredoxin and auxin responsive GH3 proteins (You et al. 2014). Flavonoid 3-monoxygenase and phenylalanine ammonia lyase-I (You et al. 2014). Elongation factor (eF-1) alpha-gene and HSP-70 Kda protein (Guo et al. 2017). Glutathione reductase (Guo et al. 2017), Oxalate-CoA-decarboxylase (Lee et al. 2009), ROS scavenging and malate dehydrogenase (Wang et al. 2018). Serine rich splicing factor (Guo et al. 2017).		

Temperature (low and high)			
Protein folding (Guo et al. 2017). Stress (Guo et al. 2017; Lee et al. 2009; Wang et al. 2018). RNA metabolism (Guo et al. 2017). Signal transduction (Guo et al. 2017; Zhang et al. 2017, 2018; Wang et al. 2015; Wang et al. 2018). DNA metabolism (Guo et al. 2017). Transport (Guo et al. 2017; Wang et al. 2015). Regulatory mechanism (Lee et al. 2009). Detoxification (Lee et al. 2009). Energy metabolism (Lee et al. 2009). Amino acid metabolism (Dumont et al. 2011). Glycolysis (Dumont et al. 2011). Pentose phosphate pathway (Dumont et al. 2011). Photosynthesis (Dumont et al. 2011). Nucleotide metabolism (Dumont et al. 2011). Secondary metabolism (Dumont et al. 2011; Wang et al. 2015). Kreb cycle (Dumont et al. 2011). Defense (Dumont et al. 2011; Wang et al. 2015). Post translational modification (Wang et al. 2018). ROS homeostasis (Zhang et al. 2017, 2018; Xin et al. 2016). Transcription factor (Zhang et al. 2017, 2018). Enzyme metabolism (Wang et al. 2015). Protein storage (Wang et al. 2015). Metabolism (Wang et al. 2015). Cell structure (Wang et al. 2015). Cell signalling (Xin et al. 2016). Protein storage (Xin et al. 2016). Calvin cycle (Xin et al. 2016).	Isocitrate dehydrogenase and beta-glucosidase (Guo et al. 2017), MAP kinase and calcium dependent protein kinase (Wang et al. 2018), BRII 1-KD-interacting protein 114 and plastid glutamine synthetaseisoform GS2C (Wang et al. 2015), ZFN like protein (Zhang et al. 2017, 2018). FACT-complex subunit (Guo et al. 2017). Glycotransferase acetyl-CoA-acetyl transferase (Guo et al. 2017), ATP synthase CFI beta subunit and ATP synthase CFI alpha subunit (Wang et al. 2015). Cysteine synthase (Lee et al. 2009). Oxalyl-CoA-decarboxylase and Glyoxalase-I (Lee et al. 2009). Pyruvate orthophosphate dikinase and putative aconitate hydrolase (Lee et al. 2009). Amino acid dehydrogenase (Dumont et al. 2011). Fructose bisphosphate aldolase-2 (Dumont et al. 2011). Ribose-5-phosphate isomerase (Dumont et al. 2011). RuBiscoactivase (Dumont et al. 2011). Nucleotide-disphosphate-kinase-2 (Dumont et al. 2011). Caffeoyl-CoA-O-methyl-transferase (Dumont et al. 2011), SAM synthase (Wang et al. 2015). Malate dehydrogenase (Dumont et al. 2011). Tyrosine kinase (Dumont et al. 2011), 2-cysteine-peroxiredoxin BAS1 and glycine decarboxylase P-subunit (Wang et al. 2015). Ubiquitin E3 (Wang et al. 2018). Chlororespiratory reduction and serine carboxypeptidase II-3 (Wang et al. 2015), Cytochrome P-450 protein and myo-inositol oxygenase 5 (Xin et al. 2016). bHLH and CoP9 protein (Wang et al. 2015). Rubiscoactivase B and Rubiscoactivase small isoform glyceraldehyde-3-phosphate dehydrogenase (Wang et al. 2015). 60-KDa chaperonin subunit beta and alpha putative chloroplast protease (Wang et al. 2015). PS16 proteins (Wang et al. 2015). Actin proteins (Wang et al. 2015). HSPs 90 and peptidylprolyl-cis trans isomerases (Xin et al. 2016). Fructose bisphosphatealdolase and phosphoglycerate kinase (Xin et al. 2016).	Post-translational modification (Pi et al. 2017). Signal transduction (Pi et al. 2017; Gao et al. 2017). Transport (Pi et al. 2017). Sugar metabolism (Gao et al. 2017). Transcription factor (Gao et al. 2017).	Phosphofructokinase and Phosphatase E-3 ligase (Pi et al. 2017). Phosphatidylinositol and MAP-K kinase (Pi et al. 2018), HY5 kinase and MAP kinase (Gao et al. 2017). P-1512 and P-1669 proteins (Pi et al. 2017). Sucrose phosphate synthase and trehalose-6-phosphate synthase (Gao et al. 2017). Serine protein kinase (Gao et al. 2017).

(continued)

Table 1 (continued)

Heavy metal stress			
Defense (Kumar and Majeti 2014). Carbohydrate metabolism (Kumar and Majeti 2014; Romeo et al. 2014; You et al. 2014). Cell signalling (Kumar and Majeti 2014). Amino acid metabolism (Kumar and Majeti 2014; Liu et al. 2014; Chen et al. 2015; Cheng et al. 2017). Energy metabolism (Kumar and Majeti 2014; Romeo et al. 2014; You et al. 2014; Cheng et al. 2017). Transport (Kumar and Majeti 2014; You et al. 2014; Xue et al. 2015; Chen et al. 2015). Redox homeostasis (Xue et al. 2015; Chen et al. 2015). Post-translational modification Romeo et al. 2014). Vacuolar dysfunction (Romeo et al. 2014). Cell wall metabolism (You et al. 2014; Cheng et al. 2017; Chen et al. 2015). Stress response (You et al. 2014; Cheng et al. 2017; Kumar and Majeti 2014). Nucleic acid metabolism (You et al. 2014). Signal transduction (Kumar and Majeti 2014; Li et al. 2016; Xue et al. 2015; Romeo et al. 2014). Fatty acid metabolism (You et al. 2014). Secondary metabolism (Liu et al. 2014). Starch and sucrose metabolism (Liu et al. 2014). TCA cycle (Liu et al. 2014). Transcriptional regulation (Chen et al. 2015). Glycosylation (Li et al. 2016). DNA binding protein (Li et al. 2016). Reservoir (Xue et al. 2015). Starch metabolism (Xue et al. 2015). Protein synthesis (Xue et al. 2015). Metabolism (Xue et al. 2015). Photosynthesis (Xue et al. 2015; Cheng et al. 2017).	Peroxidase and aldo-keto-reductase (Kumar and Majeti 2014). 2, 3-bis-phosphoglycerate-mutase-1 (Kumar and Majeti 2014), 2,3-bisphosphoglycerate mutase and succinate dehydrogenase (Romeo et al. 2014; You et al. 2014). Receptor like protein synthase and chalcone flavones isomerase (Kumar and Majeti 2014). Adenosyl-homo-cysteinase (Kumar and Majeti 2014), Peroxidase and ferrodoxin NADP-reductase (Liu et al. 2014), glutamine synthase and putative glutamine synthase (Chen et al. 2015), cysteine synthase and SAM-synthase (Cheng et al. 2017). ATP-synthase subunit beta, mitochondria and ATP-synthase subunit alpha, mitochondria (Kumar and Majeti 2014), NADH-quinoneoxido-reductase (Romeo et al. 2014), malate dehydrogenase and fructose-1,6-bisphosphatase (Cheng et al. 2017). Aquaporin NIP-1 (Kumar and Majeti 2014), Chloroplastic outer envelope membrane (Xue et al. 2015), Translation initiation factor 5A and putative elongation factor (Chen et al. 2015). L-ascorbate peroxidase1and superoxide dismutase (Xue et al. 2015), putative peroxidise and germin like protein-6 (Chen et al. 2015). Glyceraldehyde-3-phosphate dehydrogenase and fructose-bisphophatealdolase (Romeo et al. 2014). Receptor kinase and 2-oxoglutarate dioxygenase (Romeo et al. 2014). ATPase (Romeo et al. 2014). NADP-malic enzyme (You et al. 2014), UDP-arabinopyranose mutase-I (Cheng et al. 2017), Putative caffeoyl-CoA-methyl transferase (Chen et al. 2015).	Signal transduction (Zhong et al. 2017; Lan et al. 2012). Redox homeostasis (Zhong et al. 2017). Transcription factor (Zhong et al. 2017; Lan et al. 2012). Cellular component and molecular function (Zhong et al. 2017). Sugar metabolism (Zhong et al. 2017). Stress and defence (Zhong et al. 2017). Plasma membrane bound transporter (Lan et al. 2012). Carbohydrate metabolism (Lan et al. 2012).	Calmodulin dependent protein phytochelatin synthase (Zhong et al. 2017), 3-phosphoglycerate dehydrogenase (Lan et al. 2012). Mitogen activating kinase (Zhong et al. 2017). RAS kinase (Zhong et al. 2017), Putative pyrophosphatase (Lan et al. 2012). Threonine kinase (Zhong et al. 2017). Sucrose-1-related kinase (Zhong et al. 2017). Superoxide mutase (Zhong et al. 2017). Gln-synthase and phosphatidylinositol kinase (Lan et al. 2012). Calcium calmodulin-dependent kinase (Lan et al. 2012.

Cell wall related protein (Xue et al. 2015). Carbon metabolism (Romeo et al. 2014; Cheng et al. 2017). Transcription factors (Cheng et al. 2017). Protein folding (Cheng et al. 2017).	Alcohol dehydrogenase (You et al. 2014), superoxide dismutase and glutathione-*s*-transferase (Cheng et al. 2017). Transcriptional endoplasmic reticulum ATPase (You et al. 2014). Monoascorbate reductase (You et al. 2014), probable receptor like kinase (Kumar and Majeti 2014), Nucleoside diphosphate kinase and receptor activate protein kinase (Romeo et al. 2014), phosphoglycerate kinase and glutamine synthetase (Li et al. 2016), glucose and ribitol dehydrogenase (Xue et al. 2015). Maturase-K and Xanthine dehydrogenase (You et al. 2014). Putative calcium dependent protein kinase (You et al. 2014). Putative enoyl-acyl carrier protein reductase (You et al. 2014). Chloroplast–NADPH (Liu et al. 2014). Plasma membrane type ATPase (Liu et al. 2014). Glutamine synthase (Chen et al. 2015). Putative oxidase (Chen et al. 2015). CHP-rich zinc finger like protein (Chen et al. 2015). Cal-reticulin-2 (Li et al. 2016). Phosphoglycerate kinase and glutamine synthase (Li et al. 2016). Stress induced protein-1 (Li et al. 2016). Orthophosphate dikinaseRubisco (Xue et al. 2015), Ribulose bisphosphate carboxylase chlorophyll binding protein (cheng et al. 2017). Histone 2A1 protein (Li et al. 2016). Preproglutelingermin-like protein (Xue et al. 2015). ADP-glucose pyrophosphorylase and sucrose synthase 3 (Xue et al. 2015). Protein disulfideisomerase and putative ketol acid reductoisomerase (Xue et al. 2015). Phosphoglucomutase and methionine adenosyl transferase-2 (Xue et al. 2015). Reversibly glycosylated polypeptide (Xue et al. 2015). Aconitase and succinate dehydrogenase (Romeo et al. 2014), phosphoglucomutase and NADH-dehydrogenase iron sulphur protein (Cheng et al. 2017). 30-S ribosomal protein 2 and elongation factor-2 (Cheng et al. 2017). Alpha-amylase/trypsin inhibitor CYP 38 cis isomerase (Cheng et al. 2017).		

approach, the effects of abiotic stress factors on protein abundance have been examined in model, horticultural plants and crop and non-crop species such as Arabidopsis (Guo et al. 2014), rice (Paul et al. 2015, Chen et al. 2015), *Cucumis sativus* and *Solanum tuberosum* (Aghaei et al. 2008), wheat (Gao et al. 2011; Li et al. 2018), barley (Witzel et al. 2009, 2010; Mostek et al. 2015), soybean (Mustafa and Komatsu 2014), stiff grass (Cheng et al. 2017), sunroot (Zhang et al. 2017, 2018), shrubby cinquefoil (Guo et al. 2017), alfalfa (Atikur et al. 2016), Chinese grass (Xue et al. 2015), poplar (Romeo et al. 2014). Similarly, phosphoproteome studies were conducted on different crop/plants such as Arabidopsis (Bhaskara et al. 2017), rice (Zhong et al. 2017), maize (Hu et al. 2015) mulberry (Pi et al. 2017), apple (Ren et al. 2017), banana (Gao et al. 2017) and soybean (Pi et al. 2018).

2.1 Drought

Drought is a widespread environmental stress that limit agricultural productivity worldwide (Carrão et al. 2016). Despite many decades of research, drought stress is continues to be a challenging task to the agricultural scientists in general and plant breeders, in particular (Surabhi 2018). Plant response to drought has become very important in current plant biology research because it causes many changes in the biology of the plant cell, beginning with the stress perception and followed by physiological and molecular changes that promote the acclimation to the stress. Physiological processes like photosynthesis, respiration, water relations, anti-oxidative metabolism and hormonal metabolism are affected by drought (Farooq et al. 2009; Bhargava and Sawant 2013).

2.1.1 Proteome Analysis Under Drought

The proteomic studies of different species under drought stress have been extensively studied to date (Atikur et al. 2016; Paul et al. 2015; Simova-Stoilova et al. 2015a, b; Khan and Komatsu 2016; Li et al. 2018; Zadraznik et al. 2017). The altered level of expression of several protein families such as secondary metabolism, carbohydrate metabolism, energy metabolism, stress response, ROS scavenging proteins, transcription factors, signal transduction, protein folding, hormonal synthesis and cell wall metabolism have been well elucidated from different proteomic studies under drought (Ghaffari et al. 2013; Atikur et al. 2016; Paul et al. 2015; Khan and Komatsu 2016). It has been found that generation of reactive oxygen species (ROS) during drought stress can damage the structures of proteins, lipids and cell membrane integrity which ultimately destroy the plant cell (Atikur et al. 2016).

The higher amount of ROS scavenging proteins in plants increases the resistance mechanism to cope up with particular stress conditions (Atikur et al. 2016; Simova-Stoilova et al. 2015a, b). Some novel proteins which have a key role in generation of drought tolerant plants were found in the form of R40C1, cytosolic ascorbate peroxidase and putative F-box proteins during the proteomic analysis of rice and

alfalfa (Paul et al. 2015; Atikur et al. 2016). Beta-glucosidase which was found to be involved in cell wall modification during proteomic analysis of bean under drought stress showed the highest increase in the abundance during initial and final drought treatment (Zadraznik et al. 2017). Many specialized proteins are differentially expressed in plants during drought, where they have a role as signalling molecules (Atikur et al. 2016), reactive oxygen scavengers (Liu et al. 2017), proteins with responses to pathogen-related (Paul et al. 2015), heat shock proteins, late embryogenesis abundant (LEA) proteins (Liu et al. 2017) and chaperones (Liu et al. 2009; Veeranagamallaiah et al. 2011).

2.1.2 Phosphoproteome Analysis Under Drought

Phosphoproteomic studies were conducted on different plants in response to drought stress (Harb et al. 2010; Hu et al. 2015; Bhaskara et al. 2017; Ren et al. 2017). Functional analysis of maize and wheat proteins during drought stress has revealed that phosphoproteins were involved in signalling pathways or activation of receptor signalling in the form of kinases, protein transport, mRNA processing and transcription factors like bZIP-30, MYB1R1, bHLH and AB15 (Bhaskara et al. 2017; Ren et al. 2017). Phosphoproteome analysis of drought treated wheat and Arabidopsis revealed phosphorylated proteins such as ABA induced SnRK, mitogen activated protein kinases (MAPK) and calcium dependent protein kinases (Umezawa et al. 2013; Ren et al. 2017). Phosphoproteomic studies in crop/plants under drought stress are rather scanty and it requires more focus on this aspect in order to get deeper insights on stress signalling process.

2.2 Salinity

Salt tolerance is a complex phenotype which is controlled by multiple genes. Identifying novel genes, determining their expression patterns in response to salt stress and exploring their functions in stress adaptation are the basis for implementing effective engineering strategies to improve salt tolerance in plants (Cushman and Bohnert 2000). It is estimated that salt stress may affect half of all arable lands by 2050, and will be a major factor responsible for the loss of arable land for the coming decades (Wang et al. 2003a, b).

2.2.1 Proteome Analysis Under Salinity

Salinity induced tissue specific proteome studies were conducted on different crop/plants (Guo et al. 2011, 2014; Mostek et al. 2015; Witzel et al. 2014; Li et al. 2015; Zhang et al. 2017, 2018). While some proteomic studies have focused on the plant response within a few hours of encountering stress (Chitteti and Peng 2007; Li et al. 2015), others have been more interested in studying the response over a

number of days (Guo et al. 2011; Witzel et al. 2014). Functional analysis of proteins identified during salt stress in different plants were involved in protein transport, carbohydrate mechanism, ATP-synthesis, protein folding, detoxification, signal transduction, cell wall modification, energy metabolism, glycolysis, post-translational modification and defence response gives a basic insight into the mechanism of plants to cope with salt stress (Guo et al. 2011, 2014; Mostek et al. 2015; Witzel et al. 2014; Li et al. 2015; Zhang et al. 2017, 2018). During proteomic analysis of salt-sensitive and salt-tolerant barley lines revealed that enhanced salinity tolerance of barley line, that is, DH-187 observed as a result of an increased activity of signal transduction mechanism and cell wall structural changes (Mostek et al. 2015). Majority of the proteins involved in the cell wall metabolism and secondary metabolism were found to be increase in abundance in salt stressed Arabidopsis and cotton roots (Guo et al. 2014; Li et al. 2015). Witzel et al. (2014) have identified some of the new candidate proteins underlying salinity tolerance in barley, such as germin-like, pathogen related and cell-wall modification (β-1,3-glucanase) proteins (Table 1).

2.2.2 Phosphoproteome Analysis Under Salinity

Plants respond to salt stress by triggering phosphorylation cascades to turn on the salt overly sensitive (SOS) signalling pathway (Zhu 2001; Hsu et al. 2009; Jun et al. 2010; Pi et al. 2018). The phosphoproteomic studies were conducted in different plants under salt stress (Hsu et al. 2009; Jun et al. 2010; Pi et al. 2018) and signalling responses and phosphorylation cascades are suggested to function in transmitting and amplifying the extracellular salt stress signals in plants (Jun et al. 2010; Pi et al. 2018). It was found that the growth of *Thellungiella* roots was less inhibited by high-salinity stress than Arabidopsis and also *Thellungiella* roots have higher abilities to limit the Na influx than Arabidopsis because of expression of specific Na/K antiporter (Hsu et al. 2009; Jun et al. 2010). Five novel membrane proteins, that is, AHA1, STP1, patellin-2 and probable receptor kinase were identified in salt treated Arabidopsis plant (Hsu et al. 2009). Three MYB proteins were found to be differentially phosphorylated upon salt treatment in soybean and it was reported that over-expression of the GmMYB173S59D and GmCHS5 resulted in the enhancement of salt tolerance mechanism (Pi et al. 2018). The above-mentioned investigations suggested the power of proteomic and phosphoproteomic approaches in identifying functional proteins responsive to salt stress in plants. However, our understanding of salt stress responsive proteins in different tissues of crop plants is still far from complete.

2.3 Waterlogging

Waterlogging is defined as prolonged soil saturation with water at least 20% higher than the field capacity (Aggarwal et al. 2006). It is a major problem of utmost importance as it limits the growth and yield of many crops in humid areas.

Globally, approximately 10% of irrigated farmlands suffer from frequent waterlogging; however, values up to 20% occur in specific regions such as Eastern Europe and the Russian Federation (FAO 2002; Alam et al. 2010). During 1993, approximately 20 million acres of corn and soybean were inundated in the mid-western United States leading to heavy economic loss, as estimated by United State Department of Agriculture, National Agricultural Statistics Service (Suszkiw 1994). The deleterious effects associated with hypoxia and anoxia include a decrease in cellular energy charge, drop in cytoplasmic pH, and the accumulation of toxic metabolites and reactive oxygen species (ROS) which are responsible for the slowed growth and reduced yield of many agriculturally important crops (Subbaiah and Sachs 2003; Surabhi 2018).

2.3.1 Proteome Analysis Under Waterlogging

Several proteomic studies on crop/plants in responses to waterlogging (flooding) stress revealed that it affects the proteins involved in several metabolic pathways such as cellular processes, defence mechanism, secondary metabolite synthesis, protein storage and amino acid metabolism (Ahsan et al. 2007; Komatsu and Hossain 2013). Earlier studies revealed that waterlogging treatment of maize seedlings drastically altered the profile of total protein synthesis. In an anaerobic environment, 20 proteins, which account for more than 70% of the total translation, are selectively synthesized (Sachs et al. 1980). A proteomic examination of the soybean cell wall found that flooding induces a suppression of lignification through a decrease in the expression of proteins involved in ROS scavenging (Komatsu et al. 2010). In another study, it was revealed that accumulation of glycoproteins localized in the secretory pathway decreased under flood stress in soybean. Further, some novel proteins, that is, 3-β-hydroxylases, glutamyl t-RNA reductase, cysteine proteases, auxin-amidohydrolase and coprophyrinogen oxidase were identified in soybean during flooding stress (Ahsan et al. 2007; Komatsu and Hossain 2013).

2.3.2 Phosphoproteome Analysis Under Waterlogging

The effect of flooding on soybean has been extensively studied because soybean is a flood-in tolerant crop, whose growth and grain yield are significantly reduced under flooding stress (Nanjo et al. 2010, 2012). Comparative gel-free proteomics and gel-based phosphoproteomics techniques were used to investigate early responses to flooding stress in the roots and hypocotyls of soybean seedlings (Nanjo et al. 2010). De-phosphorylation of proteins involved in protein folding and synthesis was found to be one of the early responses. Different studies have suggested that the translational or post-translational control of proteins involved in protein folding and synthesis during flooding induces an imbalanced expression of proteins involved in several metabolic pathways, including carbohydrate metabolism, which may cause flooding-induced injury to the seedlings. Recently, gel-free mass spectrometry-based

proteomics techniques was used to compare protein phosphorylation states in the root tips of flooded soybean seedlings (Nanjo et al. 2012). A comparison of the proteins identified through phosphoproteomic and quantitative proteomic analyses revealed six proteins affected by flooding and showed changes in both abundance and phosphorylation status, including those involved in energy generation, protein synthesis and cell structure maintenance (Nanjo et al. 2010, 2012). It was concluded that protein phosphorylation is likely to play a major role in the regulation of pentose phosphate pathways, photosynthesis activities, pyruvate metabolism and ROS production which together contribute to stable energy supply that enhances flooding tolerance in *Kandelia candel*. Some novel phosphoproteins were identified in *Kandelia* during flood stress, that is, GSP, GxxSP and RSxS (Pan et al. 2018). Phosphoproteomic studies on different crop/plants under waterlogging are rather scanty. It requires attention to explore the specific set proteins expressed under waterlogging in order to utilizing them for crop improvement programs.

2.4 Temperature

The effects of global warming will not be limited to rising mean annual temperatures around the globe. There will also be a remarkable increase in both frequency and amplitude of severe temperature events, resulting in more extreme hot and cold days, more frequently (Neilson et al. 2010).

2.4.1 Proteome and Phosphoproteome Analysis Under High Temperature

When subjected to a high-temperature stress, plants generally respond through alterations in cell structure, cell membrane permeability, cell osmotic adjustment and photosynthetic activity (Dias et al. 2010). Guo et al. (2017) have studied proteomic changes in *Potentilla fruticosa* leaves after subjecting plants to 42 °C heat stress for 3 days, using isobaric tags for relative and absolute quantification (iTRAQ) coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS). They identified 35 up-regulated and 23 down-regulated proteins after the heat stress treatment. Those differentially abundant proteins were involved mainly in protein synthesis, protein folding and degradation, abiotic stress defence, photosynthesis, RNA process, signal transduction and other functions. Further, 58 proteins were categorized based on their sub-cellular localization mainly in the chloroplast envelope, cytoplasm, nucleus, cytosol, chloroplast, mitochondrion and cell membrane. In another study, Xu and Huang (2008) have reported that, upon imposition of heat stress, 70 protein spots were altered in at least one species. Both moderate and severe heat stress caused down-regulation of majority of proteins than up-regulated, and thermal *Agrostis scabra* roots had more up-regulated proteins than *Agrostis stolonifera* roots. Further, the mass spectrometry studies led to the identification of corresponding sequences of 66 differentially expressed protein spots. The results

suggested that up-regulation of sucrose synthase, glutathione *S*-transferase, superoxide dismutase and heat shock protein stress-inducible protein (Sti) may contribute to the superior root thermo tolerance of *A. scabra*. In addition, two isoforms of fructose-biphosphate aldolase were highly phosphorylated under heat stress as revealed by phosphoproteomic analysis, and thermal *A. scabra* had greater phosphorylation than *A. stolnifera*, suggesting that the aldolase phosphorylation might be involved in root thermo tolerance (Xu and Huang 2008).

Chen et al. (2011) have studied phosphoproteome of rice leaves after exposing plants to heat stress, and their study revealed 10 differentially expressed proteins. Analysis of the biological processes revealed that three of the variable phosphoproteins are involved in the Calvin cycle, two are part of hydrogen peroxide catabolism, two participate in ATP synthesis-coupled proton transport, one is involved in microtubule-based movement and one in cellular metabolic processes; the others have unknown functions. Heat stress induced the dephosphorylation of ribulose bisphosphate carboxylase (RuBisCo) and the phosphorylation of ATP synthase subunit-ß. This modification decreases the activities of these enzymes, but the functional significance of other phosphorylation events remains to be examined. Characterization of different candidate proteins expressed under high-temperature stress provides valuable information on their functional role and also scope for further utilization of the proteins/genes for developing high-temperature tolerant plants (Xu and Huang 2008; Guo et al. 2017; Surabhi 2018).

2.4.2 Proteome and Phosphoproteome Analysis Under Low-Temperature Stress

Low temperature, as an extreme environment, is responsible for 30–40% yield reduction in temperate growing areas (Thakur et al. 2010). The plants exposed to low-temperature stress reported to shift the thermodynamic equilibrium, when there is an increased likelihood that non-polar side chains of proteins become exposed to the aqueous medium of the cell, which can directly affect the stability and the solubility of many globular proteins. This leads to a disturbance in the stability of proteins or protein complexes, and, therefore, to a disruption of metabolic regulations. The investigation of proteome expression in different plants under chilling stress and identification of some novel proteins could be useful for better understanding the molecular basis of low-temperature stress responses in plants.

Hashimoto et al. (2009) have identified 12 number of cold stress responsive proteins from the rice root plasma membrane using a 2D-PAGE-based proteomic approach. The identified proteins were such as receptor-type protein kinase, GPI-anchored protein, leucine-rich repeat transmembrane protein kinase, water channel protein, plasma membrane integral protein, lipid transfer protein, phosphate transporter and MAP 3 K like protein kinase. In addition, cold shock protein-1 was significantly decreased in plasma membrane of rice under cold stress.

Two pea lines (*Pisum sativum* L.) with contrasted behaviours towards chilling and subsequent frost were studied by Dumont et al. (2011). Following a chilling

period, the Champagne line showed tolerant to frost, whereas, Terese line remains sensitive. Fifteen-root proteins were identified and these proteins were related to chilling response or cold acclimation. Altogether, the investigation revealed that cold acclimation is a very complex biological process that might be linked to genetic variability within the two pea species (Dumont et al. 2011).

In rice roots, a total of 27 up-regulated proteins were identified by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry or electro spray ionization-tandem mass spectrometry (ESI-MS/MS), after subjecting plants to chilling stress treatment (Lee et al. 2009). In their study, a group of novel proteins were identified including acetyltransferase, phosphogluconate dehydrogenase, NADP-specific isocitrate dehydrogenase, fructokinase, PrMC3, putative alpha-soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein and glyoxalase-1, in addition to the previously identified cold-stress responsive proteins. The identified proteins are involved in several cellular processes, including energy production and metabolism, vesicular trafficking and detoxification. Gene expression at mRNA level of some selected proteins revealed that transcription levels are not always concomitant to the translational level.

Phosphoproteomics analysis using LTQ-Orbitrap with mass spectrometry have elucidated the molecular mechanism of chilling (4 °C) tolerance in mulberry leaves (Pi et al. 2017). The result showed that 427 differentially expressed phosphoproteins were detected after 6 h of chilling, while a total of 611 phosphoproteins which were found to be significantly changed during 48 h of chilling injury. Several groups of phosphoproteins were identified in the form of protein kinases (CKII) which were responsible for the proteomic changes during chilling injury and also found to be involved in the signal transduction, protein modifications and translation process. Two phosphorylation proteins BpSIZ1 and BpICE1 found to be involved in transcription factors such as CBF/DREB during chilling stress was identified (Pi et al. 2017).

A comparative phosphoproteomic profiling of cold-sensitive Cavendish and relatively cold-tolerant Dajiao under cold stress was conducted to identify the differentially expressed proteins in banana (Gao et al. 2017). The study revealed that five phosphoproteins were differentially expressed and kinesin proteins showed a difference between the two cultivars of banana during cold stress (Gao et al. 2017). Western blot analysis showed that T31 phosphoproteins were increased, while MKK2 was decreased in Daojiao during cold stress. In case of Cavendish, MKK2 was increased, while T31 was not detected during cold stress (Gao et al. 2017). Identification of chilling related pathways and novel phosphopeptides in plants would broaden the insight into chilling response.

2.5 Heavy Metal Stress

Heavy metal pollution of air and agricultural soils is one of the most important ecological problems worldwide. Although many heavy metals occur naturally in the earth's crust at various levels, the problem arises when they are released in excess

into the environment due to natural and/or anthropogenic activities (Singh et al. 2016). Large areas of land have been contaminated with heavy metals due to the use of pesticides, fertilizers, municipal and compost wastes, and also due to heavy metal release from smelting industries and metalliferous mines (Yang et al. 2005). The annual toxicity of all toxic metals mobilized exceeds the combined total toxicity of radioactive and organic wastes produced every year from all sources (Nriagu and Pacyna 1998).

2.5.1 Proteome Analysis Under Heavy Metal Stress

Liu et al. (2014) have utilized recently developed 6-plex Tandem Mass Tag (TMT) for relative and absolute quantitation methods to achieve a comprehensive understanding of Cu tolerance/detoxification molecular mechanisms in *Elsholtzia splendens* root cell wall, for the first time. An LC-MS/MS approach was followed to analyse the Cu-responsive cell wall proteins and polysaccharides. The majority of 22 up-regulated proteins was involved in antioxidant defence pathway, cell wall polysaccharide remodelling and cell metabolism process. Changes in polysaccharide amount, composition and distribution could offer more binding sites for Cu ions. Further, the 33 down-regulated proteins were involved in signalling pathway, energy and protein synthesis.

In another study by Chen et al. (2015) have investigated the differences in Cu-binding protein expression between Cu-tolerant and Cu-sensitive rice varieties using a new IMAC method. In total, 27 differentially expressed Cu-binding proteins were identified, out of which 16 proteins were not previously identified as Cu-IMAC-binding proteins either from plants or animals (Chen et al. 2015). These novel Cu-binding proteins were of four main types, proteins involved in antioxidant defence and detoxification, putative pathogenesis-related proteins, putative cold-shock domain proteins and eukaryotic translation initiation factors.

Kumar and Majeti (2014) have studied Pb-stress effects on *Talinum triangulare* Jacq. (Willd.) after exposing the plants for 7 days and proteomic study was performed for control and 1.25 mM Pb-treated plants to examine the root protein dynamics in the presence of Pb. Twenty-three major proteins showed increased abundance, of which three proteins are new (appeared only in 1.25 mM Pb). Functional categorization of identified proteins under 1.25 mM Pb-stress have given a very clear indication about their involvement in root architecture, energy metabolism, reactive oxygen species (ROS) detoxification, cell signalling, primary and secondary metabolisms, and molecular transport systems.

The seedlings of 'Sour pummelo' (*Citrus grandis*) and 'Xuegan' (*Citrus sinensis*) were irrigated for 17 weeks with 2 μM (control) or 600 μM (Mn-toxic) MnSO4 (You et al. 2014). Two-dimensional gel electrophoresis (2-DE) subsequent analysis yielded 11 up-regulated and 42 down-regulated protein spots from Mn-toxic *C. sinensis* roots, and 25 up-regulated and 14 down-regulated protein spots from Mn-toxic *C. grandis* roots. This indicates more remarkable metabolic flexibility in *C. sinensis* roots than in *C. grandis* ones. They found important differences in

Mn-toxicity-induced changes in root protein profiles as well as root metabolic responses between the two species, especially in these proteins involved in protein biosynthesis and degradation, nucleic acid metabolism, carbohydrate and energy metabolism, and stress responses. The abundance of proteins related to nucleic acid metabolism, glycolysis and cell transport increased in non-tolerant *C. grandis* roots in response to Mn-toxicity, and decreased in tolerant *C. sinensis* roots (You et al. 2014) (Table 1).

2.5.2 Phosphoproteome Analysis Under Heavy Metal Stress

Zhong et al. (2017) have studied Cd stress effect on rice seedlings using an iTRAQ-based quantitative phosphoproteomic approach. They identified 2454 phosphosites, associated with 1244 proteins, and a total of 482 of these proteins became differentially phosphorylated under Cd stress. Number of proteins which were affected at 100 μM Cd^{2+} was sixfold higher than in 10 μM treatment. Functional analysis of the proteins which were differentially phosphorylated under stress revealed that a significant number was involved in signalling, stress tolerance and reactive oxygen species metabolism, in addition transcription factor related proteins were identified (Zhong et al. 2017). Currently, proteome and phosphoproteome analysis under heavy metal stress in crop plants is infancy and more attention is required to get deeper molecular insights of heavy metal stress tolerance in crop plants.

3 Combined Proteomics and Phosphoproteomic Studies Under Different Abiotic Stress in Crop Plants

Significant amount of proteome work has been conducted on crop/plants under different abiotic stresses. However, phosphoproteome studies in plants dealing with abiotic stresses or combined proteome and phosphoproteome studies are rather scanty. One biochemical manifestation common to all stresses is specific, regulated protein phosphorylation. It is universally accepted that a major part of the signal linking is the environmental perception of the stress at the cell surface to the nucleus, where response proteins can be translated, Protein phosphorylation is generally transmitted by protein kinase cascades (Kersten et al. 2009). A few kinase-mediated signalling pathways have been elucidated (e.g. Asai et al. 2002) in the model plants *Arabidopsis thaliana* (van Bentem and Hirt 2007; Pitzschke et al. 2009) and rice (Chen and Ronald 2011). A picture of the complexity of these signalling pathways, with all their cross-talk and branch points, is beginning to emerge. Since these pathways rely principally on post-translational modification to transmit their signal, their elucidation is well served by a proteomic approach.

Guo et al. (2014) have conducted two-dimensional gel-based proteome (coomassie brilliant blue R-350 stain) and phosphoproteome (Pro-Q diamond stain)

studies coupled with mass spectrometry to investigate salt stress induced alterations in protein profiles in the model plant, Arabidopsis roots. Non-synchronous differences were found between total proteins and phosphorylated proteins. Ten differential spots were common between 28 differential total protein spots and 13 differential phosphoproteins spots. The identified proteins are involved in binding, catalysis, signal transduction, transport, metabolisms of cell wall and energy, and reactive oxygen species (ROS) scavenging and defence (Guo et al. 2014).

Chitteti and Peng (2007) have investigated differential expression of proteins after imposing salinity stress for 24 h in rice roots. They have utilized both SYPRO ruby and Pro-Q diamond stain to study proteome and phosphoproteome fractions, respectively. Thirty-one differentially regulated proteins revealed by SYPRO ruby and 28 differentially regulated putative phosphoproteins revealed by Pro-Q diamond stain were identified using mass spectrometry. Seven proteins displayed differential expression whether the gel was stained by Pro-Q diamond or SYPRO ruby stain. The other differentially regulated proteins were specific either to Pro-Q diamond or SYPRO ruby stain, suggesting, necessity of conducting proteome and phosphoproteome studies in order to obtain holistic view of plant response to abiotic stresses (Chitteti and Peng 2007).

In another study, Lv et al. (2014) have conducted combined proteome and phosphoproteome study on *Brachypodium distachyon* leaves, after imposing salt stress. A total of 80 differentially expressed protein spots corresponding to 60 unique proteins were identified. Phosphopeptide purification was carried using TiO_2 micro-columns and LC-MS/MS for phosphoproteome analysis to identify phosphorylation sites and phosphoproteins. A total of 1509 phosphoproteins and 2839 phosphorylation sites were identified. Among them, 468 phosphoproteins containing 496 phosphorylation sites demonstrated significant changes at the phosphorylation level. Of the 60 unique differentially expressed proteins, 14 were also identified as phosphoproteins. Many proteins and phosphoproteins, as well as potential signal pathways associated with salt response and defence, were found, including three 14-3-3s (GF14A, GF14B and 14-3-3A) for signal transduction and several ABA signal-associated proteins such as ABF2, TRAB1 and SAPK8. Based on different studies, it is clear that the overlapping between proteome and phosphoproteome within different studies under varying stress conditions were found minimal. Therefore, it necessitates conducting both proteome and phosphoproteome in each study to identify metabolic and signalling proteins, respectively, under abiotic stress in crop plants.

4 Mass Spectrometry in Proteomic and Phosphoproteomic Studies

The technology of choice for proteomics is mass spectrometry (MS) including several approaches such as liquid chromatography–mass spectrometry (LC-MS/MS), ion trap–mass spectrometry (IT-MS) and matrix-assisted laser desorption/

ionization–time of flight mass spectrometry (MALDI-TOF-MS) (Komatsu and Hossain 2013; Shao et al. 2015). However, it is necessary to choose the appropriate instrument for the purpose as there is no MS that can be useful for all fields of proteome analysis. MALDI-TOF/MS is often used for high-throughput identification of the protein by peptide mass fingerprinting (Witzel et al. 2009, 2010). In the analysis of amino acid sequence and post-translational modification, MS/MS such as ESI-IT and ESI-Q-TOF/MS are used. These technologies are basically used in measuring the mass and charge of small protein fragments (or 'peptides') that result from protein enzymatic digestion with special enzymes called proteases, such as trypsin (Helmy et al. 2012a; Nakagami et al. 2012). The output of a standard MS-based proteomic analysis is a set of peptide fingerprints called MS spectra. MS spectra require another layer of interpretation to reveal the peptide sequences associated with each of them, the protein of each peptide and the modification occurring in each protein after being translated (Tyers and Mann 2003; Helmy et al. 2012a, b; Nakagami et al. 2012).

Proteomic and phosphoproteomic investigation was carried on plants under different abiotic stress conditions by using different mass spectrometry platforms such as MALDI-TOF/MS (Atikur et al. 2016; Paul et al. 2015), LC-MS/MS (Zadraznik et al. 2017; Li et al. 2018; Guo et al. 2017), LC-ESI-MS/MS (Ren et al. 2017; Zhang et al. 2017, 2018), nano-LC-MS/MS (Oskuei et al. 2017; Zhang et al. 2017, 2018; Li et al. 2018; Wang et al. 2018), nano-LC-ESI-Q-TOF-MS/MS (Pan et al. 2018), nano-LC-ESI-LIT-MS/MS (Romeo et al. 2014) and nano-RPLC-MS/MS (Pi et al. 2018). Despite these technological innovations and advancements, the analysis of a full proteome is still a challenging task, mainly because of the high complexity of protein samples (Bachi and Bonaldi 2008; Surabhi 2018). To overcome this difficulty, several separation techniques such as multi-dimensional chromatography, MudPit (Washburn et al. 2001) or specific enrichment/depletion techniques, tandem affinity purification (Gavin et al. 2002) and equalizer beads (Guerrier et al. 2008) can be applied prior to mass spectrometric analysis. These approaches increase the proteome coverage and the dynamic range of large-scale proteomics analysis.

4.1 Gel-Based Proteomic and Phosphoproteomic Analysis in Plant Abiotic Stress

2-DE coupled with MALDI-TOF-MS or ESI-Q-TOF-MS/MS are the most common technique used in the abiotic stress-related proteomic studies. 2-DE resolves proteins on the basis of isoelectric point (pI) and molecular mass (Mr) (Roy et al. 2011). The separated protein spots can then be stained, with coomassie brilliant blue, silver nitrate, or SYPRO Ruby (Robinson et al. 2011), among others. When combined with advanced MS techniques, 2-DE allows hundreds of proteins to be characterized in a single polyacrylamide gel (Magdeldin et al. 2014), including the position of the protein spot (pI and Mr) on the gel. This capability of 2-DE has allowed for analysis of post-translational modifications (PTMs) of proteins. Two-dimensional gel-based proteomic and phosphoproteomic analysis were conducted on plants

under different abiotic stresses such as drought (Atikur et al. 2016; Paul et al. 2015; Simova-Stoilova et al. 2015a, b), salt (Chitteti and Peng 2007; Jun et al. 2010; Guo et al. 2014; Wen et al. 2014; Witzel et al. 2014; Mostek et al. 2015), heavy-metal (Romeo et al. 2014; You et al. 2014), low-temperature (Lee et al. 2009) and water-logging stress (Alam et al. 2010). The DIGE technique was developed to improve the reproducibility of 2-DE and to overcome gel-to-gel variation (Unlu et al. 1997). Each protein sample is labelled at a lysine residue with different fluorophores, such as CyDye2, CyDye3 and CyDye5 (Beckett 2012), prior to mixing and separation on the same gel, and the abundance of the same protein in different samples can easily be determined by using these fluorophores (Magdeldin et al. 2014). This technique reduces the number of gels needed for one experiment and is able to detect as little as 150 pg of a single protein with a linear response in protein concentration of over five orders of magnitude. Differential-in-gel electrophoresis (DIGE) performed in different plants in response to several abiotic stress such as salt (Gao et al. 2011) and heavy-metal stress (Kumar and Majeti 2014; Chen et al. 2015; Xue et al. 2015; Cheng et al. 2017). The relatively high cost of DIGE equipment, software and consumables, however, has limited its use. Despite the successes of 2-DE, the method has many limitations (Robinson et al. 2011). For example, 2-DE can separate only 30–50% of the entire proteome, depending on the tissue, and it is unable to separate all the proteins present in a complex sample (Beckett 2012).

The low-abundance proteins with physiological relevance, including regulatory and signal-transducing proteins or phosphoproteins, are also rarely detected on traditional 2-DE gels, because the large amount of highly abundant proteins masks their detection (Roy et al. 2011). For instance, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), which accounts for a large percentage of total plant protein, hinders absorption of low-abundant proteins on the immobilized pH gradient (IPG) strips and results in poor detection and identification of these proteins on 2-D gels and by MS (Beckett 2012). Different staining technique have applied for visualization of proteins and phosphoproteins which were differentially expressed under different abiotic stress such as colloidal coomassie blue R-250, R-350 and G-250 (Chen et al. 2015; Paul et al. 2015; Simova-Stoilova et al. 2015a, b; Xue et al. 2015; Atikur et al. 2016; Cheng et al. 2017) and SYPRO-ruby staining (Chitteti and Peng 2007). The Pro-Q diamond in gel stain was found to be useful method for direct visualization of the putative phosphoprotein spots expressed under different abiotic stress such as salt (Chitteti and Peng 2007; Jun et al. 2010; Guo et al. 2014; Liu et al. 2014) and drought (Yuan et al. 2016).

4.2 Gel-Free Proteomics and Phosphoproteomics Analysis in Plant Abiotic Stress

In iTRAQ (Isobaric Tags for Relative and Absolute Quantitation), samples are labelled at peptide level and it is an LC-based gel-free method. All the proteins present in requisite amounts will be systematically quantified and identified in iTRAQ

method, and at the end it provides a more comprehensive map of the protein content of a sample (Alvarez et al. 2009). iTRAQ labelling overcomes some of the limitations of 2-D gel-based techniques and also improves the throughput of proteomic studies. This technique has a high degree of sensitivity, and the amine specific isobaric reagents of iTRAQ allow the identification and quantitation of up to eight different samples simultaneously (Ross et al. 2004; Aggarwal et al. 2006; Zieske 2006). iTRAQ can identify proteins outside the pH range of commonly used gels and distinguish between proteins that would co-migrate on a gel, whereas DIGE resolves only soluble proteins included in a pH range of 3–11 (Alvarez et al. 2009). iTRAQ-based proteomics and phosphoproteomic analysis have conducted in plants in response to different abiotic stresses, that is, drought (Hu et al. 2015; Ren et al. 2017; Sun et al. 2017), salt (Zhang et al. 2017, 2018; Pi et al. 2018), heavy metal (Lan et al. 2012; Zhong et al. 2017) and high temperature (Guo et al. 2017; Wang et al. 2018). Recent advancement in LC-MS-based quantitative techniques such as isotope-coded affinity tags (ICAT) (Gygi et al. 1999), stable isotope labelling by amino acids in cell cultures (SILAC) (Schutz et al. 2011), and isobaric tags for relative and absolute quantification (iTRAQ) (Alvarez et al. 2009) showed advantages for relative quantification of proteins or peptides on a large scale. Advances in these techniques and in the MS field can allow the analysis of complex proteomes at organ/tissue and whole plant levels in different crops. This technological advancement in gel-free proteomics could further expand our scope of understanding of abiotic stress sensing mechanisms in plants.

Immobilized metal ion affinity chromatography (IMAC) is a common separation platform used prior to MS analysis for large-scale identification of protein phosphorylation sites from complex samples (Nühse et al. 2003). Typically, phosphopeptides are bound by immobilized metal ions through metal-phosphate affinity interactions, and non-phosphorylated peptides are removed by washing. The phosphopeptides can be released from the solid support by phosphate or alkaline elution. Several metal ions were employed for IMAC, and each metal ion has distinct strengths and weaknesses (Zhou et al. 2008). Among these metal ions, Fe^{3+} is the most common metal ion used in the IMAC approach; however, its specificity is insufficient for comprehensive phosphoproteome analysis (Kinoshita et al. 2004). IMAC-based phosphoproteomics analysis has been conducted in *Arabidopsis thaliana,* banana, rice and chickpea in response to salinity (Hsu et al. 2009), low-temperature (Gao et al. 2017) and heavy metal stress (Lan et al. 2012; Chen et al. 2015). Metal affinity chromatography (TiO_2)-based phosphoproteomic studies were conducted on banana and *Ammopiptanthus mongolicus* under low-temperature (Gao et al. 2017) and drought (Sun et al. 2017). IMAC and LC-MS/MS-based phosphoproteomics analysis on *Arabidopsis thaliana* during salt stress has revealed that level of phosphopeptides on five membrane proteins such asAHA1, STP1, Patellin-2, probable inactive receptor kinase (At3g02880) and probable purine permease-18 showed at least twofold increase in comparison to control in response to 200 mM salt-stress (Hsu et al. 2009).

5 Conclusion

Investigating the molecular events occurring in stress responses using gel-based and gel-free phosphoproteomic studies will enhance our understanding of the biological processes in crop plants. Recent advancement in proteomic methodologies, such as multi-dimensional protein fractionation (MudPit), SILAC, ICAT, iTRAQ, IMAC, DIGE and high-resolution tandem mass spectrometry, has facilitated a more accurate comparison of crop stress responses and can detect more deferentially abundant proteins than prior analysis. Sensitive proteomic approaches are capable of identifying low-abundance proteins (especially transcription factors and regulatory proteins) involved in the initial stress response in crops. Currently, majority of the crop proteomic changes often analysed after several hours, even days after a stress onset. A focus on early responsive proteins is required in order to identify regulatory and signalling proteins. Combined proteome and phosphoproteome analysis of the response of plants to stress at the protein and phosphoprotein level, together with physiological measurements, will assist in identifying the novel proteins and pathways that are crucial for stress tolerance. Further, proteomics has identified a vast number of proteins that participate in the growth of plants or their adaptation to environmental stresses. Functional analysis of those proteins will contribute to the development of high-yielding crops through artificial manipulation of the basic life phenomena of plants or through the assessment of their stress tolerance. In addition, integration of proteomics result with findings from other large-scale 'omics' and bioinformatics applications will surely facilitate the establishment of molecular networks underlying abiotic stress response and tolerance in crop plants.

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