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## Abstract

Major abiotic stress that limits plant growth and agriculture productivity is the soil salinity. In order to minimize the detrimental effects of salinity, highly complex salt-responsive signaling and metabolic processes at the cellular, organ, and whole-plant levels have been evolved in the plants. Currently, it has become the need of the hour to understand the molecular basis of salt stress signaling and tolerance mechanisms in cereals for engineering and/or screening for more tolerance to salt stress. Valuable information will be provided through investigation of the physiological and molecular mechanisms of salt tolerance for effective engineering strategies. Current advancement in proteomics has helped us in studying the sophisticated molecular networks in plants. Reports of proteomics studies about plant salt response and tolerance mechanisms, especially that of cereals, have revealed the mechanisms that include changes in photosynthesis, scavenging system of reactive oxygen species (ROS), ion homeostasis, osmotic homeostasis, membrane transport, signaling transduction, transcription, protein synthesis/turnover, cytoskeleton dynamics, and cross talks with other stresses.

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## Introduction

Salinity, one of the most significant abiotic stresses, not only limits the productivity and geographical distribution of plants but also causes ion imbalance, hyperosmotic stress, and oxidative damage in plants leading to molecular damage,

growth and yield reduction, and even plant death (Wang et al. 2004). Infiltration and accumulation of NaCl (Tuteja 2007) is the major cause of salinization and can result in soil Na<sup>+</sup> concentration above 40 mM which can suppress the growth of most crops (Wong et al. 2006). Salt accumulation has been attributed to the natural phenomena and human activities like irrigation. About 1/5 of the earth's arable land and 50% of the irrigated one are under salinity (Mahajan and Tuteja 2005; Munns and Tester 2008). Plants have coped with this problem through various sophisticated mechanisms that include selective ion

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uptake/exclusion, compartmentalization of toxic ions, synthesis of compatible products, adjustment of photosynthetic and energy metabolism, accumulation of antioxidative enzymes, regulation of hormones, and modification of cell structure. Molecular and physiological aspects of plant salt stress tolerance have been revealed through physiological, molecular genetics, and functional genomics studies. A few important genes responsible for the above-stated mechanisms have been cloned, and their involvement in plants' response and adaptation to salinity has been confirmed (Tuteja 2007).

However, state-of-the-art transcriptomics studies have helped us in the collection of immense data on their expression at the mRNA level (Chand and Kumar 2006; Diedhiou et al. 2009; Jha et al. 2009; Wong et al. 2005, 2006; Zhang et al. 2001b, 2008; Zouari et al. 2007), and these data present a global vision about salt-responsive genes in different plants. But mRNA levels do not usually correlate with the expression levels of proteins due to posttranscriptional events and posttranslational modifications such as phosphorylation and glycosylation. These proteins are more directly related to signaling and metabolic processes under salt stress conditions. So the need of the hour is to study the salt tolerance mechanisms at the so-called protein level which is possible through the optimum utilization of proteomic technologies.

The plant taxa have an extensive genetic diversity for salt tolerance as it is distributed over numerous genera (Flowers and Colmer 2008) making them either glycophytes (salt-sensitive or hypersensitive plants) or halophytes (native flora of saline environments). In some halophytes, very special anatomical and morphological adaptations or avoidance mechanisms have been employed (Flowers and Colmer 2008), but on the basis of these, we are unable to introgress the responsible genes into crop plants. During the last decade, it has been established that most halophytes and glycophytes use analogous tactical processes rather than similar strategies to tolerate salinity (Hasegawa et al. 2000b). For example, cytotoxic ions ( $\text{Na}^+$  and  $\text{Cl}^-$ ) are compartmentalized into the vacuole

and used as osmotic solutes under saline environments (Blumwald et al. 2000; Niu et al. 1995). It follows that there is similarity among many of the molecular entities that mediate ion homeostasis and salt stress signaling in all plants (Hasegawa et al. 2000b) as, for example, ion homeostasis that facilitates plant salt tolerance resembles that described for yeast (Bressan et al. 1998; Serrano et al. 1999). According to another finding of genomics, there is remarkable colinearity (gene synteny and homology) of gene sequences among different grass species, including cultivated crops (Tang et al. 2008; Zahn et al. 2008), and strongly suggests their resemblance in evolution and probably in functions. The facts stated above have made it feasible to use a model system for the dissection of the plant salt stress response (Bressan et al. 1998; Hasegawa et al. 2000a; Sanders et al. 1999; Serrano et al. 1999; Zhu 2000, 2001b). Since a salt-tolerant genetic model is required for complete delineation that if salt tolerance is affected most by form or function of genes or more by differences in the expression of common genes either due to transcriptional or posttranscriptional control (Zhu et al. 2007). In this way, our understanding of cellular salt tolerance mechanisms has been greatly increased through research on the plant genetic model, the *Arabidopsis*, a glycophyte. It will also enable us to effectively apply the genetic learning of one crop to another and also will yield large spillover benefits from investment in such research, like possible eventual interspecies gene transfers.

Till now, more than 2,171 salt-responsive proteins/enzymes have been discovered in different parts of 34 plant species like shoots, leaves, roots, seedlings, radicles, hypocotyls, grains, gametophytes, and unicells (Zhang et al. 2012). Most of the research has been conducted on plants like *Arabidopsis thaliana* (Jiang et al. 2007; Kim et al. 2007; Lee et al. 2004; Ndimba et al. 2005; Pang et al. 2010) and *Oryza sativa* (Abbasi and Komatsu 2004; Cheng et al. 2009; Chitteti and Peng 2007; Dooki et al. 2006; Kim et al. 2005; Li et al. 2010; Nohzadeh et al. 2007; Parker et al. 2006; Ruan et al. 2011; Salekdeh et al. 2002; Wen et al. 2010; Yan et al. 2005;

Zhang et al. 2009), *Triticum durum* (Caruso et al. 2008), *Triticum aestivum* (Huo et al. 2004; Jacoby et al. 2010; Peng et al. 2009; Wang et al. 2008a), *Hordeum vulgare* (Rasoulnia et al. 2010; Sugimoto and Takeda 2009; Witzel et al. 2009, 2010), *Zea mays* (Zörb et al. 2004, 2009, 2010), *Setaria italica* (Veeranagamallaiah et al. 2008), *Sorghum bicolor* (Kumar et al. 2011), and *Agrostis stolonifera* (Xu et al. 2010).

This chapter includes the reports of proteomics studies about plant salt response and tolerance mechanisms, especially that of cereals, and future perspectives in food security. These mechanisms include changes in photosynthesis, scavenging system of reactive oxygen species (ROS), ion homeostasis, osmotic homeostasis, membrane transport, signaling transduction, transcription, protein synthesis/turnover, cytoskeleton dynamics, and cross talks with other stresses.

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## Role of Cereals in Food Security

The word cereal has been derived from *Ceres*, the name of the Roman goddess of harvest and agriculture. The common cereal crops include rice, wheat, corn, barley, sorghum, millet, oats, and rye. It also includes flours, meals, breads, and alimentary pastes or pasta. Cereals, the poor man's meat, provide staple food in almost every country and region as in the world as a whole; about 95% of starchy staple food comes from cereals and only 5% from root crops (mainly cassava, potato, and yams, depending on climate). Currently, about 50% of the world's cropland is under the cultivation of cereals. These provide about two-thirds of all human calorie intake, if we combine their direct intake (e.g., as cooked rice or bread) with their indirect consumption, in the form of foods like meat and milk (about 40% of all grain is currently fed to livestock).

Among cereals, wheat is the predominant commodity consumed for food accounting 68% of total cereal use. By 2020, total cereal consumption is projected to reach nearly 746 metric tons (Mt) (145 Mt wheat, 529 Mt rice, and the

rest maize, barley, etc.) with per capita food consumption of around 66 kg per person per annum, and 2% of world wheat is utilized for biofuel production; ultimately the total consumption is expected to increase from 68 to 75% (Chand and Kumar 2006).

According to Chand and Kumar (2006), to feed a population of eight billion by 2025, average world cereal yield of about 4 metric tons/ha will be required (Evans 1998). In addition, it has been found out the reasons for food insecurity that include increasing demand from growing population, climate change, and increased linkages between energy and agricultural commodities due to the growing demand for biofuels (Chand and Kumar 2006). The emerging food insecurity condition will prevail mostly in developing countries, especially that of Asia. However, more than 90% of rice and 43% of wheat in the world is produced and consumed in Asia.

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## Detrimental Effects of Salinity on Cereals

Salinity is one of the major environmental factors that adversely affect the crop growth and development processes like seed germination (Dash and Panda 2001); seedling growth (Mahajan and Tuteja 2005; Tuteja 2007; Wang et al. 2004; Wong et al. 2006); enzyme activity (Seckin et al. 2009); deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein synthesis (Anuradha and Rao 2001); mitosis (Tabur and Demir 2010); vegetative growth (Hamed et al. 2007; Panda and Khan 2009); and flowering and fruit set (Zhu 2001a, b) and ultimately result in diminished economic yield and also quality of produce.

Since sensitivity or tolerance to salt stress is different in all plant species (Ashraf and Harris 2004), so plants are classified into two groups, namely, glycophytes or halophytes, on the basis of their ability to grow on high-salt medium. Most of the grain crops and vegetables are glycophytes and cannot tolerate salt stress as high salt concentrations decrease the osmotic potential of soil solution creating water stress in plants

and ion toxicity (e.g.,  $\text{Na}^+$ ,  $\text{Cl}^-$ ) since  $\text{Na}^+$  is not readily sequestered into vacuoles as in halophytes. Ultimately, it results in nutrient imbalances and their deficiencies which can lead to plant death as a result of growth arrest and molecular damage (McCue and Hanson 1990). In addition, salinity causes oxidative stress due to the production of induced active oxygen species (Heidari 2009; Munns and Tester 2008) which disrupt the cellular metabolism through oxidative damage to membrane lipid, proteins, and nucleic acids (Mittler 2002).

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## Salinity Tolerance, Multigenic Trait

Salinity is a quantitative trait, and a large number of salt-induced genes have been isolated which are concomitantly up- and downregulated (Bohnert et al. 1995). According to Meyer et al. (1990), in *M. crystallinum*, more than a 100 genes are induced, and probably transcripts, three times that number, are repressed in response to salt stress. Salinity tolerance is a mutagenic trait due to the fact that sublethal salt stress conditions cause an osmotic effect that is similar to that brought about by water deficit and to some extent by cold as well as heat stresses (Almoguera et al. 1988). So there is a high degree of similarity between salt and dehydration stress with respect to their physiological, biochemical, molecular, and genetical effects (Cushman et al. 1992). The ways in which different plants confer salt resistance are represented in the following figure (Fig. 5.1):

There is a diverse expression pattern of salt-responsive genes found in different plants (especially glycophytes and halophytes) under different salinity conditions (e.g., salt concentration and treatment time) (Nito et al. 2007). The expression pattern of genes in cereals indicates that most of the proteins related with photosynthesis in *O. sativa* (Abbasi and Komatsu 2004; Kim et al. 2005; Parker et al. 2006), *T. durum* (Caruso et al. 2008), and *T. aestivum* (Huo et al. 2004; Peng et al. 2009) and proteins involved in carbohydrate and energy metabolism in *O. sativa* (Abbasi and Komatsu 2004; Chitteti and Peng

2007; Dooki et al. 2006; Kim et al. 2005; Li et al. 2010; Nohzadeh et al. 2007; Parker et al. 2006; Ruan et al. 2011), *T. aestivum* (Peng et al. 2009; Wang et al. 2008a), *H. vulgare* (Rasoulnia et al. 2010; Witzel et al. 2010), *Z. mays* (Zörb et al. 2004, 2010), and *S. bicolor* (Kumar et al. 2011) are induced by salinity.

Most of the salt-responsive proteins are involved in basic metabolic processes like photosynthesis, energy metabolism, ROS scavenging, and ion homeostasis which make halophytes highly efficient in photosynthetic and energy metabolism, ion exclusion/compartmentalization, compatible product synthesis, induction of antioxidative enzymes and hormones, as well as modification of cell structure. Also evolution of different salt tolerance mechanisms have been found during the study of the specific proteins and/or their expression patterns in different halophytes.

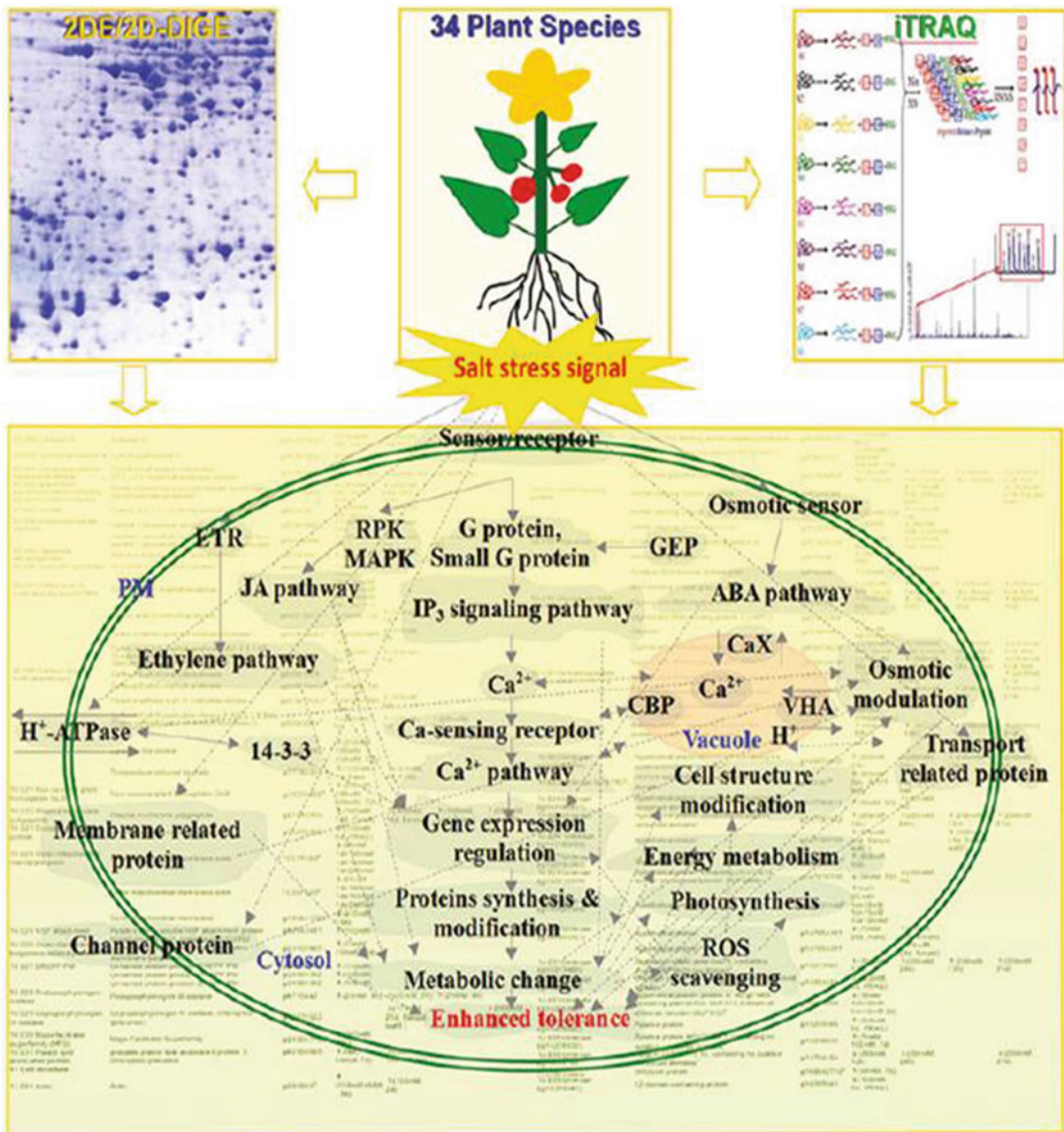
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## Molecular Mechanisms of Salinity Tolerance in Cereals

### Photosynthesis

In addition to osmotic, ionic, and nutrient imbalances in plants, salinity also disturbs the plant water uptake and biosynthesis of abscisic acid (ABA) in leaves (Fricke et al. 2004) which affects the stomatal conductance. This in turn affects the photosynthetic electron transport and the enzyme activities for carbon fixation during dark reaction (Parida and Das 2005; Tuteja 2007). Several genes involved in photosynthesis (encoding chlorophyll a-/b-binding proteins (CAB), ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), and RuBisCO activase (RCA)) have been isolated and characterized through previous studies which are directly or indirectly involved in salinity tolerance (Wong et al. 2006; Zhang et al. 2001a, b, 2008).

Currently, through the advancement in the field of proteomics, our understanding of the photosynthetic processes underlying salinity response and tolerance has been greatly enhanced. About 367 photosynthesis-related IDs, representing 26 UPs,



**Fig. 5.1** Different ways of salt tolerance in different plants (Zhang et al. 2012)

regulated by salinity in 27 plant species have been discovered (Nito et al. 2007). These are involved in the regulation of photosynthetic processes like that of light reaction, CO<sub>2</sub> assimilation, and other photosynthesis-related processes. Out of 26 UPs in all, 12 are related with light reaction involved in light-harvesting phase, proton gradient formation, electron transfer, and energy production.

In case of cereals, the concentration of photosystem II chlorophyll-binding protein 47 (CP47) and 33 kDa oxygen evolving complex (OEC) protein in *O. sativa* (Abbasi and Komatsu 2004; Kim et al. 2005; Sengupta and Majumder 2009) is increased under salinity stress which helps in the protection of reaction center proteins (D1 protein) from stomatal protease digestion and



ensures optimal functioning of photosystem II (PSII) (Enami et al. 1997). Salinity stress affects the abundance of cytochrome b6f complex involved in the transfer of electrons from PSII to photosystem I (PSI) in *Z. mays* (Zörb et al. 2009) and PSI reaction center protein in *H. vulgare* (Rasoulnia et al. 2010). The variation in the abundance of cytochrome b6f complex disturbs the electron transfer efficiency and transmembrane electrochemical proton gradients and ultimately affects ATP synthesis and NADPH formation. Adjustment of ATP synthesis and thermal dissipation take place in halophytes due to the fact that multiple isoforms of chloroplast ATP synthases (Bandehagh et al. 2011; Caruso et al. 2008; Chen et al. 2011; Huo et al. 2004; Katz et al. 2007; Kim et al. 2005; Li et al. 2011; Liska et al. 2004; Pang et al. 2010; Parker et al. 2006; Sobhanian et al. 2010a,b; Wang et al. 2008b; Yu et al. 2011; Zörb et al. 2009) and ferredoxin NADP(H) oxidoreductases (FNR) (Bandehagh et al. 2011; Caruso et al. 2008; Li et al. 2011; Liska et al. 2004; Pang et al. 2010; Peng et al. 2009; Tanou et al. 2009; Wakeel et al. 2011; Xu et al. 2010; Yu et al. 2011; Zörb et al. 2009) are regulated by salinity.

In addition to the effects of salinity on light reaction, there are also reports about the changes in the expression pattern of 14 Calvin cycle-related enzymes. During this process, CO<sub>2</sub> is fixed in chloroplasts by RuBisCO to produce 3-phosphoglycerates (PGA). The enzymes (RuBisCO-binding protein (RBP) and RuBisCO activase (RCA), FBP, SBPase, PRK, and the other five enzymes (phosphoglycerate kinase (PGK), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), triose-phosphate isomerase (TPI), fructose-bisphosphate aldolase (FBA), and transketolase (TK))) involved in this process are all affected by salinity stress and show diverse changes in different plant species (Zhang et al. 2012). Photosynthesis-related proteins affected by salt stress revealed through proteomics studies include low-CO<sub>2</sub>-inducible protein (LCIC) red chlorophyll catabolite reductase (RCCR) in *D. salina* and thylakoid lumen (TL) proteins in *T. halophila* and *A. thaliana*.

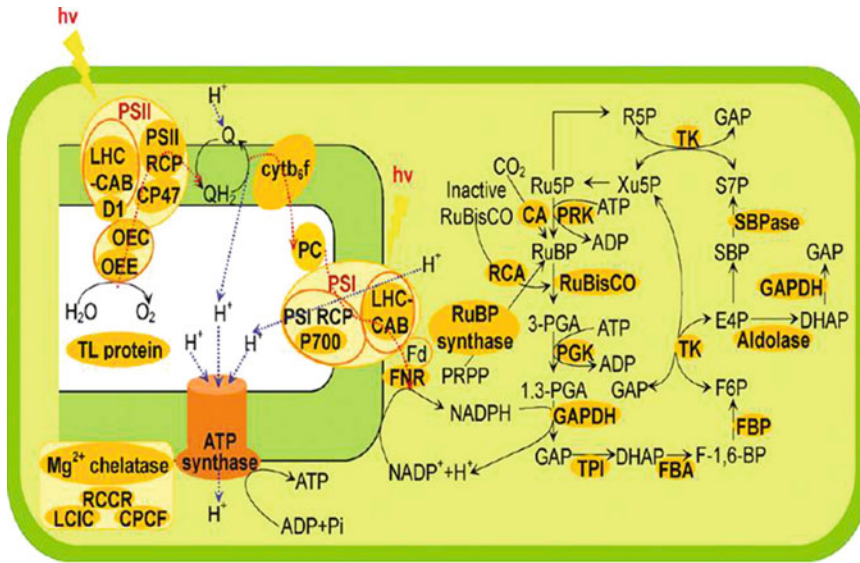
Low-CO<sub>2</sub>-inducible protein (LCIC) is found to be induced in *D. salina* when subjected to

solution of 3 M NaCl (Yu et al. 2011). LCIC, a component of the inorganic carbon transport system in the plasma membrane, is involved in carbon-concentrating mechanism, hence important for salt tolerance in the algae *D. salina*. But the concentration of two thylakoid lumen (TL) proteins, TL18.3 and TL19, found in *T. halophila* (Pang et al. 2010) and *A. thaliana* (Kim et al. 2007), respectively, is decreased under salinity stress. TL18.3 is involved in the regulation of D1 protein turnover and the assembly of PS II monomers into dimers (Sirpio et al. 2007), while TL19 is a member of PS I subunit III and is involved in the oxidation of plastocyanin in the electron transport chain (Hippler et al. 1989).

Another protein, red chlorophyll catabolite reductase (RCCR) in *C. aurantium*, is also found to be induced when treated with NaCl (Tanou et al. 2009). During chloroplast breakdown, it helps in the conversion of an intermediary red chloroplast catabolite (RCC) into primary fluorescent catabolites (pFCCs) (Rodoni et al. 1997; Takamiya et al. 2000). Its absence causes leaf cell death due to the accumulation of RCC which leads to the production of singlet oxygen (Pruzinska et al. 2007). So the increased concentration of RCCR helps in combating the problem of reactive oxygen species (ROS) and thus plays an important role in salinity tolerance. The graphical view of salinity-responsive proteins involved in the photosynthesis in plants is given in Fig. 5.2.

## ROS Scavenging System

Due to the salinity stress, the metabolites in various processes like electron transport chain in mitochondria and chloroplasts, photorespiration, fatty acid oxidation, and various detoxification reactions, cell wall peroxidases, germin-like oxalate oxidases, and amine oxidases in the apoplast are over-reduced (Miller et al. 2010; Mittler et al. 2004). This over-reduction causes the production of reactive oxygen species (ROS) like superoxide radicals (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and hydroxyl radicals (OH<sup>-</sup>). These ROS disturb cellular redox homeostasis and cause oxidative



**Fig. 5.2** Schematic representation of salinity-responsive proteins involved in the photosynthesis in plants (Zhang et al. 2012)

damage to many cellular components and structures (Jithesh et al. 2006; Parida and Das 2005; Zhang et al. 2001a, b).

To cope with this problem, plants need to activate the ROS scavenging system for enhanced salt tolerance (Zhang et al. 2001a, b). Through the advancement in the field of proteomic, scientists have discovered 184 protein IDs (representing 12 UPs) as ROS scavenging-related proteins, and most of them (143 IDs) are induced by salinity in 24 plant species (Nito et al. 2007). These proteins take part in various chemical reactions like superoxide dismutation, glutathione-ascorbate cycle, catalase (CAT) pathway, peroxiredoxin/thioredoxin (PrxR/Trx) pathway, and glutathione peroxidase (GPX) pathway (Fig. 5.3). Superoxide dismutases (SOD) is the main enzyme in ROS scavenging system, usually induced by salinity, and enhances the timely dismutation of superoxide into oxygen and  $H_2O_2$ , which is subsequently removed through different pathways.

### Glutathione-Ascorbate Cycle

One of the most important antioxidant protection systems for removing  $H_2O_2$  generated in cytosol, mitochondria, chloroplast, and peroxisomes is the glutathione-ascorbate cycle (Hasegawa et al.

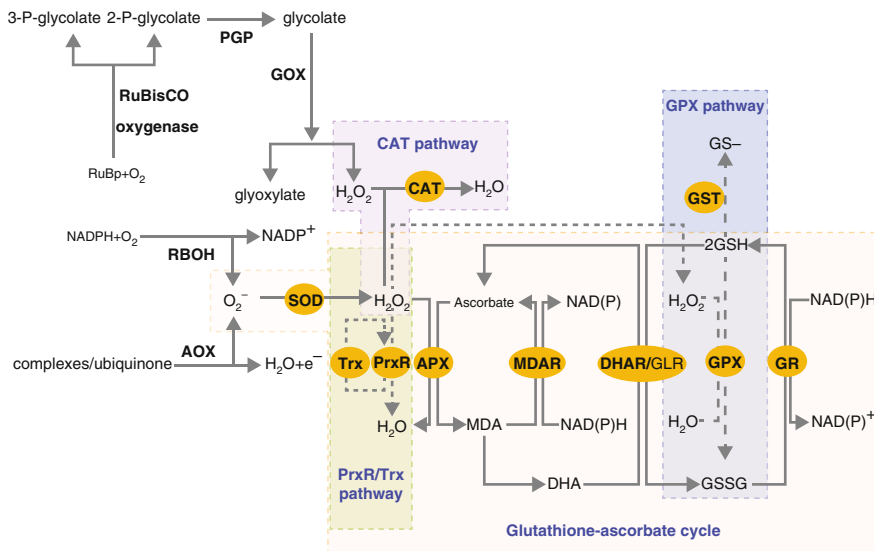
2000a; Sanders et al. 1999) in which  $H_2O_2$  is reduced to water by an enzyme ascorbate peroxidase (APX). In this process ascorbate (AsA) acts as the electron donor and is oxidized. As oxidized AsA (monodehydroascorbate, MDA) is still a radical, so it is converted into dehydroascorbate (DHA) spontaneously or by monodehydroascorbate reductase (MDAR). Various scientists have found that the concentration of APX, DHAR, MDAR, and GR is increased in *O. sativa* under salinity (Dooki et al. 2006; Li et al. 2010; Ruan et al. 2011; Salekdeh et al. 2002).

### Catalase (CAT) Pathway

CAT pathway mainly occurs in peroxisomes and helps in the reduction of  $H_2O_2$  to  $H_2O$ . Through proteomics studies, it has been found that salinity stress increases the CAT levels *O. sativa* (Kim et al. 2005; Li et al. 2010) but decreases *H. vulgare* (Witzel et al. 2009).

### Peroxiredoxin/Thioredoxin (PrxR/Trx) Pathway

It is a vital antioxidant defense system in plants in which peroxiredoxins (PrxRs), a multigenic family, and thioredoxins (Trxs) are involved in ROS metabolism (Horling et al. 2003). PrxRs utilize a thiol-based catalytic mechanism to



**Fig. 5.3** Schematic presentation of the salinity-responsive proteins/enzymes in ROS scavenging system in plants (Zhang et al. 2012)

reduce  $\text{H}_2\text{O}_2$  and are regenerated using Trxs as electron donors (Dietz 2011). Advancement in the field of proteomics has revealed that salinity affects the two main proteins, that is, PrxRs (Askari et al. 2006; Caruso et al. 2008; Chattopadhyay et al. 2011; Du et al. 2010; Kim et al. 2007; Ndimba et al. 2005; Pang et al. 2010; Peng et al. 2009; Rasoulnia et al. 2010; Sobhanian et al. 2010b; Wang et al. 2008b, 2009; Yu et al. 2011; Zörb et al. 2010) and Trxs (Du et al. 2010; Lee et al. 2004; Nohzadeh et al. 2007; Sobhanian et al. 2010a; Wang et al. 2007), of this pathway. In *Z. mays*, the concentration of PrxRs is increased (Zörb et al. 2010).

### Glutathione Peroxidase (GPX) Pathway

This pathway is one of the major mechanisms of ROS scavenging system (Yoshimura et al. 2004) in which GPX can reduce  $\text{H}_2\text{O}_2$  to the corresponding hydroxyl compounds using GSH and/or other reducing equivalents. The concentration of GPXs is increased in *S. europaea* (Li et al. 2011), while reduced in *S. aegyptiaca* (Askari et al. 2006) under salt stress conditions.

Another group of enzymes, glutathione S-transferases (GSTs), have GPX activity and can use glutathione (GSH) to reduce organic hydroperoxides of fatty acids and nucleic acids to the corresponding monohydroxy alcohols (Cummins et al. 1999; Roxas et al. 1997). These GSTs prevent the degradation of organic hydroperoxides to cytotoxic aldehyde derivatives and hence protect plants from oxidative damage under salt stress (Dixon et al. 2002). From the proteomic studies, it has been revealed that most of the GSTs are increased in salt-stressed plants (Chattopadhyay et al. 2011; Chen et al. 2011; Chitteti and Peng 2007; Jain et al. 2006; Jiang et al. 2007; Kim et al. 2007; Kumar et al. 2011; Liska et al. 2004; Pang et al. 2010; Peng et al. 2009; Rasoulnia et al. 2010; Ruan et al. 2011; Sobhanian et al. 2010b; Sugimoto and Takeda 2009; Tanou et al. 2009; Wang et al. 2008a, 2009; Witzel et al. 2009; Yu et al. 2011) but decreased in *H. vulgare* (Rasoulnia et al. 2010; Witzel et al. 2009). In addition, it has been found that the level of peroxidases (PODs) increases in all salt-stressed plants but reduces in *O. sativa* (Cheng et al. 2009; Zhang et al. 2009). PODs also play an important role in ROS scavenging system.



## Osmotic Homeostasis

As mentioned earlier, physiological water deficit and osmotic stress are the main effects of salinity in plants. In order to maintain the osmotic homeostasis, plants tend to accumulate osmolytes such as proline, soluble sugars, and glycine betaine (GB). GB is a major osmolyte which not only stabilizes the protein quaternary structure and highly ordered membrane state but also reduces lipid peroxidation during salinity stress (Chen and Murata 2008; Chinnusamy et al. 2006; Wang et al. 2004). There are a variety of proteins which are involved in osmotic homeostasis. For example, late embryogenesis abundant (LEA) proteins function to protect the steady structure of proteins, membranes, and cells (Chinnusamy et al. 2006), and their expression is increased in roots and hypocotyls of salt-treated *O. sativa* (Li et al. 2010). In *T. aestivum*, cold-regulated proteins and cold-responsive group-3 LEA/RAB-related COR proteins are induced under stress conditions (Caruso et al. 2008). Moreover, in *O. sativa* panicles, the level of an ABA-/salt-responsive 40 kDa protein, Osr40c1s, is increased in response to salt stress (Dooki et al. 2006). This protein consists of 151 amino acids in a duplicated domain which have the ability to form amphiphilic  $\alpha$ -helical structures that associate with membrane proteins for salt tolerance (Moons et al. 1997).

## Salt Stress Signal Transduction

Ionic signaling, osmotic signaling, detoxification signaling, and signaling to coordinate cell division and expansion are all included in salt stress signaling (Zhu 2002). For salt tolerance in plants, the signal transduction is a burning issue, and several salt-responsive signaling pathways have been predicted which include salt overly sensitive (SOS) signaling pathway, ABA signaling pathway,  $\text{Ca}^{2+}$  signal transduction pathway, protein kinase pathway, phospholipid pathway,

ethylene signaling pathway, and jasmonate acid (JA)-induced signaling pathway (Cao et al. 2008; Darwish et al. 2009; Mahajan et al. 2008; Zhu 2001a, b, 2002).

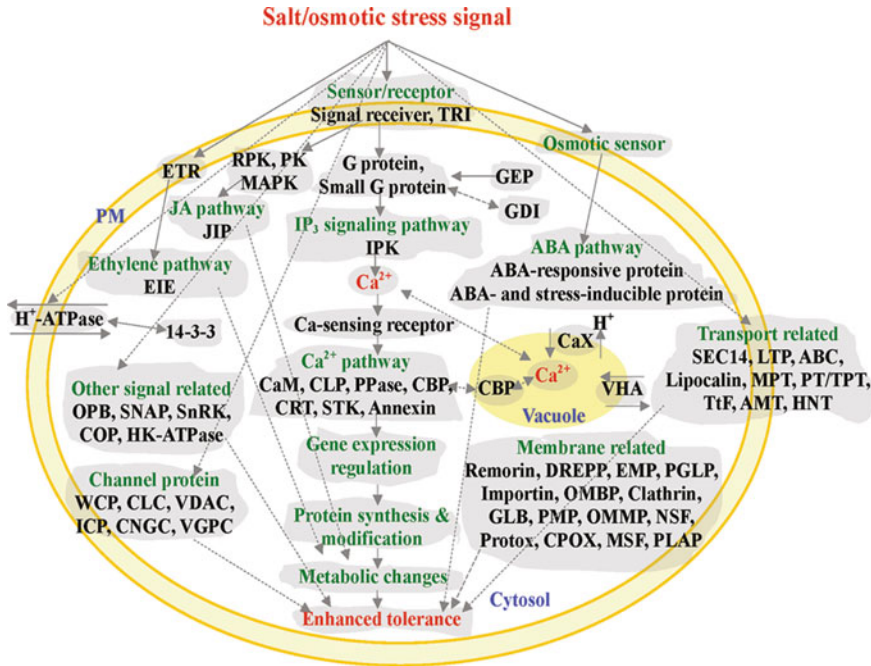
According to Tuteja (2007) and Zouari et al. (2007), advancement in proteomic research has identified about 85 IDs (24 UPs) as signal transduction-related proteins in response to salt stress (Fig. 5.4). The details of the signaling pathways mentioned above are given as follows.

### G-Protein-Coupled Receptors

Under salinity conditions, kinase-mediated protein phosphorylation and/or G-proteins are involved in the transduction of stress signals (e.g., ions, ROS, and ethylene), perceived by their receptors/sensors, and helped in the regulation of corresponding signaling and metabolic pathways. From the current proteomic research, it has been clear that there are two types of receptors (the ethylene receptor (ETR) and a transforming growth factor (TGF)-beta receptor-interacting protein) which are induced in *T. aestivum* under salinity stress (Peng et al. 2009). In addition, there are also reports about the stimulation of some of G-proteins/small G-proteins and three isoforms of receptor protein kinase (RPK) identified from *T. aestivum* (Peng et al. 2009) and *O. sativa* (Dooki et al. 2006; Zhang et al. 2009) under saline conditions. The above-stated facts suggest that ethylene and ABA signaling pathways may be involved in salt response (Cao et al. 2008). In addition to the above-stated receptors, G-protein-coupled receptors are dynamically regulated to cope with salinity. It has been found through the proteomic studies that the reduced levels of a signal receiver and G-proteins/small G-proteins in *T. aestivum* (Peng et al. 2009) and *O. sativa* (Chitteti and Peng 2007), as well as two abundance-changed guanine nucleotide exchange proteins (GEP) are involved in small GTPase activation in *O. sativa* (Chitteti and Peng 2007).

### Abscisic Acid (ABA) Signaling Pathway

ABA signaling pathway is an endogenous messenger which helps in controlling plant water status and osmotic stress tolerance through its



**Fig. 5.4** Graphical representation of different identified signaling pathways and transport-related proteins under salinity (Zhang et al. 2012)

role in guard cell regulation (Christmann et al. 2006; Zhu 2002). Cis-regulatory elements (i.e., ABA-responsive elements, ABREs) are shared by ABA-inducible genes which are involved in the regulation of plant stress responses. But it has been found that osmotic stress-responsive genes may be ABA independent, ABA dependent, or partially ABA dependent (Zhu 2002). Due to this, salt-/osmotic-responsive genes have been considered as either early response genes or delayed-response genes as these and their expression products are unknown. From the proteomic studies, it has been found that the level of several ABA-related proteins such as ABA-responsive proteins (ABR17 and ABR18) and ABA-/stress-inducible proteins (ASR1) is increased in *O. sativa* (Salekdeh et al. 2002) under salinity stress. But from the genetic analysis of ABA-deficient mutants, it has been found that ABA signaling is necessary in stomatal control of water loss (Schroeder et al. 2001). In conclusion, ABA signaling pathways are activated in the plants to protect themselves from water deficit associated with salinity.

### Jasmonic Acid (JA), Ethylene (ET), and Salicylic Acid (SA) Signaling Pathways

Through the advancement in proteomics studies, it has been found that a salt-inducible ethylene receptor in *T. aestivum* (Peng et al. 2009; Wang et al. 2008a), salt-responsive jasmonate-inducible proteins in the roots of *H. vulgare* (Witzel et al. 2009), as well as many members of pathogenesis-related (PR) protein in *O. sativa* (Li et al. 2010; Zhang et al. 2009) and *H. vulgare* (Sugimoto and Takeda 2009) serve as negative regulators of SA and positive regulators of JA-activated gene expression (Zhu 2002). There are also reports about the involvement of mitogen-activated protein kinase (MAPKs) in the integration of SA- and JA-dependent signals to evoke appropriate responses against pathogens and other stresses.

Moreover, some PR proteins (AtPR12) induced by JA/ET signaling, AtPR1 upregulated by SA signaling (Koomneef et al. 2008), as well as OsPR10 induced by JA/ET but suppressed by SA signaling in response to high salinity have also been found to be affected by JA/ET or SA

signaling. So it has been clear that powerful applications of proteomics have helped us in unraveling the molecular mechanisms underlying hormone signaling in salt tolerance.

### Ca<sup>2+</sup>/Calmodulin (CaM) Signaling Pathway

It is calcium (Ca<sup>2+</sup>)-dependent signaling network that has been reported to mediate Na<sup>+</sup> homeostasis and salt resistance in many crop plants (Mahajan et al. 2008). For example, in *Z. mays* chloroplast, a Na<sup>+</sup> sensing element and Ca<sup>2+</sup> sensing receptor are reported to be induced at 25 mM NaCl for 1 h but decreased after 4 h (Zörb et al. 2009). Calcium-binding proteins (CBPs) are regulated by salinity in *O. sativa* (Li et al. 2010). Moreover, salinity regulates the dynamics of calmodulin in *O. sativa* (Yan et al. 2005) and in *Zea mays* (Zörb et al. 2010) and that of calcineurin-like phosphoesterase in *T. aestivum* (Peng et al. 2009) and in *O. sativa* (Li et al. 2010). These changes modulate the levels of intracellular Ca<sup>2+</sup> and induce specific protein kinase/phosphatase systems (Jiang et al. 2007). So Ca<sup>2+</sup> signaling network is closely related to the activation of the SOS signal transduction pathway, which is responsible for cellular Na<sup>+</sup>/K<sup>+</sup> homeostasis and the osmolytes accumulation (Zhu 2002).

### 14-3-3 Proteins

*14-3-3 proteins* are a group of proteins which are commonly found and are multifunctional regulators of many cellular signaling pathways. This group of proteins interacts with a number of signaling molecules like calcium-dependent protein kinase (CDPK) and mitogen-activated protein kinase (MAPK). By interacting with the C terminus which is essential for the control of ion transport and cytoplasmic pH, these proteins act as positive regulators of plasma membrane (PM) H<sup>+</sup>-ATPase (Palmgren 1998). These proteins are also known to be involved in response to salinity at multiple levels including regulating target proteins with functions including signaling, transcription activation, and defense and also working as components of transcription factor complexes associated with ABA-induced gene

expression. Through the advancement in proteomics, it has been found that in *T. aestivum* (Wang et al. 2008a), *O. sativa* (Cheng et al. 2009; Nohzadeh et al. 2007), and *Z. mays* (Zhu 2003), many members of this group such as 14-3-3 protein (gi13928452, and gi12229593) (Jain et al. 2006; Zörb et al. 2010), 14-3-3-like protein (gi1168189, and gi7267542) (Wang et al. 2008a), GF14a(XP-48289), GF14b (gi50924768) (Nohzadeh et al. 2007), and GF14 kappa isoform (gi30698122) (Ndimba et al. 2005) are regulated by salinity conditions. So we can conclude that 14-3-3 proteins regulate multiple pathways involved in salt stress response.

### Ion Homeostasis and Cross-Membrane Transport

Under salinity conditions, aqueous and ionic thermodynamic equilibrium is altered through high apoplastic levels of Na<sup>+</sup> and Cl<sup>-</sup> which results in hyperosmotic stress, ionic imbalance, and toxicity. So to cope with this problem, plants reestablish cellular ion homeostasis by regulating ion uptake/exclusion and in vivo compartmentalization. Ion (e.g., K<sup>+</sup> and Na<sup>+</sup>) homeostasis, a fine-tuned process, mainly relies on the proton-motive forces created by the action of H<sup>+</sup>-ATPases, various ion channels, and transporters (Gong et al. 2001). From the recent studies, it has been found that for the regulation of the activity of plasma membrane H<sup>+</sup>-ATPases, phototropin and 14-3-3 proteins have to work cooperatively as well as independently for opening and closing of the ion channels (e.g., K<sup>+</sup> channel) (Inoue et al. 2005; van den Wijngaard et al. 2005). Currently, various researchers through proteomic studies have found that salinity stress significantly affects H<sup>+</sup>-ATPase (Barkla et al. 2009; Cheng et al. 2009; Du et al. 2010; Jiang et al. 2007; Katz et al. 2007; Manaa et al. 2011; Nat et al. 2004; Ndimba et al. 2005; Pang et al. 2010; Wang et al. 2008a, 2009; Yu et al. 2011; Zörb et al. 2004), ATP-binding cassette (ABC) transporter (Wang et al. 2008a, b, 2009), and other ion channels and transporters (Li et al. 2011; Peng et al. 2009; Sobhanian et al. 2010b; Wakeel et al.

2011; Wang et al. 2008a, b, 2009; Zörb et al. 2010).

### H<sup>+</sup>-ATPase

For the maintenance of ion homeostasis in plant cells, H<sup>+</sup>-ATPases are one of the most important enzymes because most of the vacuolar H<sup>+</sup>-ATPases in glycophytes like *O. sativa* (Cheng et al. 2009), *T. aestivum* (Wang et al. 2008a), and *Z. mays* (Zörb et al. 2004) are induced under salinity stress. For Na<sup>+</sup> transport by salt overly sensitive (SOS1) which is essential for salt tolerance, it has been that the level of H<sup>+</sup>-ATPases is increased which provides a more strong driving force for this transport (Kim et al. 2007; Zhu 2001a, b, 2003). The proton electrochemical gradient for vacuolar Na<sup>+</sup>/H<sup>+</sup> antiporter to compartmentalize Na<sup>+</sup> in the vacuoles is generated through vacuolar H<sup>+</sup>-ATPases, the major H<sup>+</sup>-pumps on the tonoplast (Chinnusamy et al. 2005). For Na<sup>+</sup> sequestration and osmotic adjustment under salinity stress, increased levels and/or activities of the vacuolar H<sup>+</sup>-ATPases are found to be a cost-effective strategy (Pang et al. 2010). Moreover, it has also been found that mitochondrial-/chloroplast-located H<sup>+</sup>-ATPases are also involved in ion homeostasis (Huo et al. 2004; Kim et al. 2005; Ndimba et al. 2005).

### ABC Transporters and Other Transporters

In *T. aestivum*, it has been found that ABC transporters (the in charge of transporting of stress-related secondary metabolites, such as alkaloids, terpenoids, polyphenols, and quinines (Yazaki 2006)) are induced under salinity stress (Wang et al. 2008a; Peng et al. 2009). There are three proteins which cause abundant changes in plants under salinity and include ferritin (Chen et al. 2009; Parker et al. 2006; Wang et al. 2009), iron deficiency-induced protein (IDI), and iron deficiency-specific protein (IDS) (Witzel et al. 2009). The first protein, ferritin, through the Fenton reaction, helps in the sequestration of excess free irons and prevents formation of hydroxyl radicals (Laohavisit et al. 2010; Parker et al. 2006) and is reported to be induced in *O. sativa* (Parker et al. 2006) under certain salinity

conditions. But in contrast, the level of other two transporters, that is, IDIs and IDSs, is decreased in *H. vulgare* under salinity stress (Witzel et al. 2009) which is beneficial in avoiding excessive ion uptake.

### Ion Channel Proteins

To maintain ion homeostasis under salinity stress, the level of different ion channels is changed, that is, increases or decreases; for example, the level of voltage-gated potassium channels in *T. aestivum* (Peng et al. 2009) is induced which is crucial for a balance of K<sup>+</sup>/Na<sup>+</sup> in the cells, but in case of a cyclic nucleotide-gated ion channel (CNGC), the level is reduced as a nonselective cation channel (Wang et al. 2008a). The direct binding of cyclic nucleotides (cAMP and cGMP) helps in the opening of CNGC of which the activity is of little voltage dependence, but Ca<sup>2+</sup>/calmodulin and phosphorylation help in its modulation. Another ion channel, annexin, revealed through proteomic studies, is a Ca<sup>2+</sup>-permeable channel at endomembrane and plasma membrane for the formation of a ROS-stimulated passive Ca<sup>2+</sup> transport pathway (Laohavisit et al. 2010), and its level is reported to be induced in various cereal crops like *O. sativa* (Li et al. 2010) and *T. aestivum* (Peng et al. 2009). Its increased level plays a vital role in osmotic adjustment and subsequently cell expansion and exocytosis (Faurobert et al. 2007; Lee et al. 2004). Moreover, it has been found that the level of voltage-dependent anion channel protein (VDAC), a barrel protein located at the outer mitochondrial membrane and responsible for passage of small molecules (<1,000 Da) into the intermembrane space, is induced under salinity stress in *Z. mays* (Sugimoto and Takeda 2009). Its dynamic changes are found to influence the mitochondrial respiration (Rostovtseva and Bezrukov 2008; Rostovtseva et al. 2008).

### Plasma Membrane and Other Membrane-Associated Proteins

Twenty four plasma membrane proteins are reported to be found in rice which are induced under salinity stress and regulate the development

of plasma membrane polypeptides containing a Glu-rich site at the C terminus, responsible for calcium binding in  $\text{Ca}^{2+}$  signal transduction pathway (Yuasa and Maeshima 2000). In addition, a plant-specific PM/lipid-raft protein, remorin, helps in maintaining the membrane skeletons (Bariola et al. 2004) and thus contributes in the stabilization of damaged PM under salinity stress (Cheng et al. 2009; Nohzadeh et al. 2007). Moreover, the level of importin (a nuclear membrane transporter) and an outer mitochondrial membrane porin in *T. aestivum* (Peng et al. 2009; Wang et al. 2008a; Witzel et al. 2009) is reduced under salinity stress.

### Transcription and Protein Fates

Changes in transcriptional regulatory networks of cis-/trans-elements and transcription factors can be triggered through aforementioned signaling systems. Through the advancement in proteomic research, it has been found that the levels of transcription factors and transcription-related proteins are regulated under salinity stress and play a vital role in salt tolerance (Zhang et al. 2012). To cope with salt stress in *T. aestivum* (Peng et al. 2009; Wang et al. 2008a), the salt-induced transcription factor, basic transcription factor 3 (BTF3), is an important regulatory component and controls diverse processes. In addition, the increased levels of DNA polymerases in *Z. mays* (Zörb et al. 2004) and DNA helicases in *T. aestivum* (Wang et al. 2008a) enhance DNA replication, unwinding, and transcription under salinity. Furthermore, there are also reports about the effects of salinity on some RNA processing- and splicing-related proteins such as maturase K (Chattopadhyay et al. 2011; Wang et al. 2009; Zörb et al. 2010), nucleic acid-binding proteins (Aghaei et al. 2008; Bandehagh et al. 2011; Caruso et al. 2008; Chen et al. 2009; Jain et al. 2006; Kim et al. 2005; Pang et al. 2010; Tanou et al. 2009; Wang et al. 2008b; Witzel et al. 2009), glycine-rich RNA-binding proteins (Askari et al. 2006; Dooki et al. 2006; Jiang et al. 2007; Manaa et al. 2011), and RNA splicing factors (Wang et al. 2008a; Yan et al. 2005). In abiotic stress adaptation, protein synthesis plays a very

important role, and proteomics studies have found that many of its components including different ribosomal proteins (Aghaei et al. 2008; Bandehagh et al. 2011; Chattopadhyay et al. 2011; Chen et al. 2009; Chitteti and Peng 2007; Dani et al. 2005; Du et al. 2010; Jiang et al. 2007; Kim et al. 2005; Ndimba et al. 2005; Pang et al. 2010; Peng et al. 2009; Sobhanian et al. 2010a, b; Veeranagamallaiah et al. 2008; Wang et al. 2008b; Zörb et al. 2004, 2010), translation initiation factors (Jiang et al. 2007; Ndimba et al. 2005; Pang et al. 2010; Parker et al. 2006; Peng et al. 2009; Wang et al. 2008a, b, 2009; Yu et al. 2011), poly(A)-binding proteins (Jiang et al. 2007; Witzel et al. 2009), translation elongation factors (Chen et al. 2009; Liska et al. 2004; Ndimba et al. 2005; Pang et al. 2010; Peng et al. 2009; Sobhanian et al. 2010b; Witzel et al. 2010), translationally controlled tumor proteins (Nat et al. 2004; Pang et al. 2010; Sobhanian et al. 2010b; Vincent et al. 2007; Witzel et al. 2010; Yu et al. 2011; Zörb et al. 2010), RNA recognition motif (RRM)-containing proteins (Pang et al. 2010; Yu et al. 2011), and tRNA synthases (Ndimba et al. 2005; Pang et al. 2010; Peng et al. 2009; Wen et al. 2010) are altered in expression under salinity stress conditions.

Generally, salinity stress represses protein synthesis (Tuteja 2007). However, the level of some of the above proteins is increased which shows that normal cellular processes are required for the maintenance of protein synthesis activities under salinity stress (Zhang et al. 2012). For maintaining normal cellular functions under salinity stress, correct protein folding and transport is crucial. For this, heat shock proteins (HSPs) and other molecular chaperons play a very important role in protein structure stabilization and subcellular localization (Vierling 1991). Salinity affects various HSPs/chaperonins (Aghaei et al. 2008; Chattopadhyay et al. 2011; Chen et al. 2009, 2011; Chitteti and Peng 2007; Dani et al. 2005; Du et al. 2010; Geissler et al. 2010; Jain et al. 2006; Jiang et al. 2007; Katz et al. 2007; Kim et al. 2005, 2007; Li et al. 2010; Liska et al. 2004; Manaa et al. 2011; Nat et al. 2004; Ndimba et al. 2005; Pang et al. 2010; Peng et al. 2009; Razavizadeh et al. 2009; Sengupta and Majumder 2009;

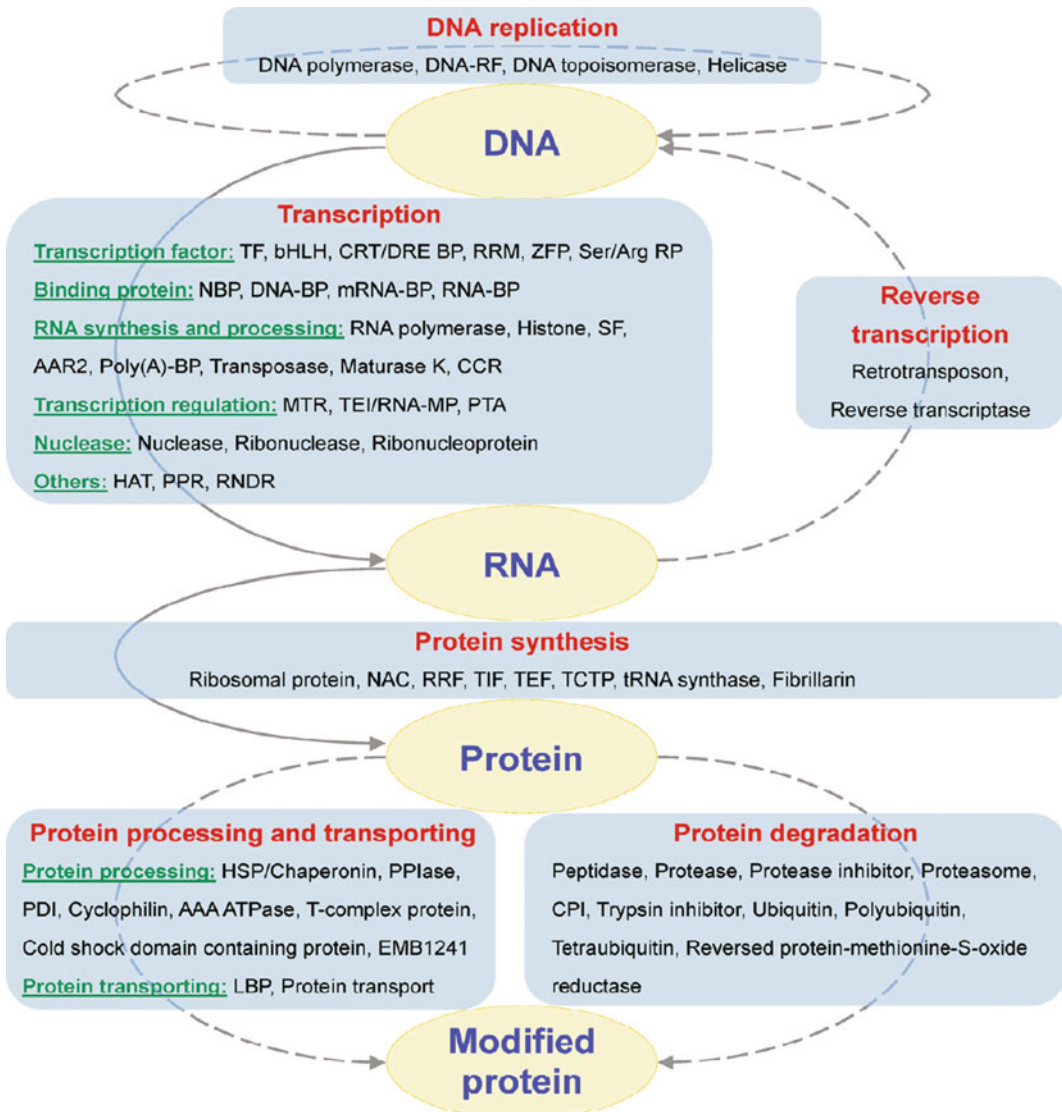


Sobhanian et al. 2010a, b; Tanou et al. 2009; Wang et al. 2008a, b, 2009; Wen et al. 2010; Witzel et al. 2010; Xu et al. 2010; Yu et al. 2011; Zörb et al. 2010), luminal-binding proteins (LBP) (Kim et al. 2007; Liska et al. 2004; Pang et al. 2010; Wang et al. 2008b, 2009), peptidyl-prolyl cis-trans isomerases (Aghaei et al. 2008; Askari et al. 2006; Chen et al. 2011; Tanou et al. 2009; Zörb et al. 2009), protein disulfide isomerases (PDI) (Chen et al. 2011; Jiang et al. 2007; Nohzadeh et al. 2007; Pang et al. 2010; Yu et al. 2011), T-complex proteins (Pang et al. 2010; Wang et al. 2008a), AAA ATPase superfamily proteins (Chen et al. 2011; Wen et al. 2010; Yu et al. 2011), and cold shock domain-containing proteins (Huo et al. 2004; Peng et al. 2009; Ruan et al. 2011) which are involved in maintaining normal protein folding, repair, and renaturation of the stress-damaged proteins (Fig. 5.5). In addition, for the selective degradation of proteins, plants use proteasome pathways. Some members of these pathways such as ubiquitin/poly-ubiquitin/tetraubiquitin (Du et al. 2010; Katz et al. 2007; Xu et al. 2010), SKP1 protein (Liska et al. 2004), proteasome components (Askari et al. 2006; Chattopadhyay et al. 2011; Jiang et al. 2007; Kim et al. 2005; Liska et al. 2004; Peng et al. 2009; Sobhanian et al. 2010b; Tanou et al. 2009; Vincent et al. 2007; Wang et al. 2008a, b, 2009), various proteases (Katz et al. 2007; Liska et al. 2004; Manaa et al. 2011; Pang et al. 2010; Peng et al. 2009; Veeranagamallaiiah et al. 2008; Wang et al. 2008b, 2009; Xu et al. 2010; Yu et al. 2011; Zörb et al. 2009;) and peptidases (Chitteti and Peng 2007; Jiang et al. 2007; Li et al. 2010; Liska et al. 2004; Manaa et al. 2011; Ndimba et al. 2005; Pang et al. 2010; Wang et al. 2008b; Yu et al. 2011), protease inhibitors (Aghaei et al. 2009; Chen et al. 2011; Peng et al. 2009; Sobhanian et al. 2010a), and reversed protein-methionine-S-oxide reductases (Pang et al. 2010) under salinity stress exhibit tremendous changes. Degradation of proteins not only is important in protein turnover during ubiquitin-mediated degradation of proteins but also helps in the regulation of other cellular processes such as signal transduction

and transcription. So, these salt-responsive proteins have a vital role to play in salinity tolerance.

## Cytoskeleton and Cell Structure

For cell turgor maintenance, a rapid remodeling of cytoskeleton for the adjustment of cell size takes place during salinity stress (Pang et al. 2010; Li et al. 2011). Through the advancement in the field of proteomics, it has been found that basic cytoskeleton components such as actin (Cheng et al. 2009; Jiang et al. 2007; Li et al. 2010; Tanou et al. 2009; Xu et al. 2010) and tubulin (Jiang et al. 2007; Katz et al. 2007; Kim et al. 2007; Liska et al. 2004; Pang et al. 2010; Peng et al. 2009) and other cytoskeleton-related proteins (some actin-binding proteins (ABPs)) (Yan et al. 2005), kinesin motor (Chitteti and Peng 2007; Sobhanian et al. 2010a; Wang et al. 2009), myosin (Cheng et al. 2009; Peng et al. 2009; Wang et al. 2009), and xyloglucan endotransglycosylase (XET) hydrolases (Zörb et al. 2010) are changed under salinity stress. ABPs such as actin-depolymerizing factors (ADFs) (Sobhanian et al. 2010b), profilins (Askari et al. 2006; Du et al. 2010; Wang et al. 2009), and cyclase-associated proteins (CAPs) (Ndimba et al. 2005) play key roles in the remodeling as these have the ability to bind with actin cytoskeletons; for example, ADFs promote filamentous actin disassembly and thus modulate the dynamic organization of actin cytoskeletons. Profilin has the ability to join with actin monomers and cause polymerization/depolymerization of actin filaments to maintain cell structure integrity, cell mobility, tumor cell metastasis, and growth factor signaling (Staiger et al. 1997). Similarly CAPs which are multifunctional ABPs are involved in various signal transduction pathways involved in cell growth, development, vesicle trafficking, and endocytosis. Under salinity stress, it has been found that cytoskeleton dynamics is associated with other physiological



**Fig. 5.5** Schematic representation of the salinity-responsive proteins involved in transcription and protein metabolism (Zhang et al. 2012)

changes like the osmotic stress regulation of actin organization that correlates well with  $K^+$  channel activity in guard cells (Luan 2002). For controlling cell expansion and morphology, tubulins may co-migrate with P-type ATPases (Campetelli et al. 2005) or connect with the plasma membrane (Drykova et al. 2003).

Moreover, XETs are involved in cutting and rejoining of intermicrofibrillar xyloglucan chains and help in wall loosening and ultimately for cell expansion. So under salinity stress, growth inhibition occurs due to reduced expression of aforementioned enzymes (Fry et al. 1992).

## Cross-Tolerance to Multiple Stresses

Various signaling and metabolic pathways are connected into networks because plants have developed cross-tolerance mechanisms to cope with different stresses at the same time (Tuteja 2007); for example, in salt tolerance, some biotic stress-responsive proteins/genes play important roles. Carbohydrate-binding proteins like lectins are involved not only in defense against predators and pathogens (De Hoff et al. 2009) but also in plant salt tolerance (Van Damme et al. 2004). Researchers through proteomics studies have found that salinity stress enhances not only the levels of lectins in *O. sativa* (Chitteti and Peng 2007) and *S. bicolor* (Kumar et al. 2011) but also that of cytoplasmic mannose-binding lectins (Claes et al. 1990; Hirano et al. 2000; Zhang et al. 2000) and salt stress-induced proteins (salt proteins) (Chitteti and Peng 2007; Wen et al. 2010). The biotic stress-related proteins, revealed through proteomics, include elicitor peptides (Aghaei et al. 2009; Wang et al. 2009), disease-related/resistance proteins (Chattopadhyay et al. 2011; Chitteti and Peng 2007; Nat et al. 2004; Peng et al. 2009; Wang et al. 2008a, 2009), hypersensitive-induced response proteins (Cheng et al. 2009; Nohzadeh et al. 2007), pathogenesis-related proteins (Chattopadhyay et al. 2011; Jain et al. 2006; Li et al. 2010; Manaa et al. 2011; Pang et al. 2010; Sugimoto and Takeda 2009; Vincent et al. 2007; Wang et al. 2008b, 2009; Zhang et al. 2009), stress-inducible proteins (Dooki et al. 2006; Wang et al. 2008a; Witzel et al. 2009), and universal stress protein family (Kumar et al. 2011; Li et al. 2010; Ndimba et al. 2005; Wang et al. 2008a), and while that of abiotic stress-related proteins include cold-regulated proteins (Wang et al. 2009), cold-responsive LEA-/RAB-related COR proteins (Caruso et al. 2008), and copper homeostasis factors (Jiang et al. 2007). Salt tolerance is also contributed by the multifunctional glyoxalase system. Under salinity stress, plants tend to accumulate high amounts of methylglyoxal (MG) which is a by-product of glycolysis mainly

from triose phosphate. MG has detrimental effects on plants as it involved in the inhibition of cell proliferation (Ray et al. 1994), degradation of proteins, and inactivation of antioxidant defense system (Martins et al. 2001). MG is detoxified through glyoxalase system consisting of glyoxalase I (GlyI) and glyoxalase II (GlyII). From the previous molecular studies involving transgenic plants, it has found that overexpression of GlyI and GlyII enhances plant salt tolerance (Singla-Pareek et al. 2003, 2008; Yadav et al. 2005). From the proteomics, it has been found that the level of GlyI increases in *O. sativa* (Chitteti and Peng 2007; Li et al. 2010) when treated with 150 mM NaCl for 6 or 48 h (Jiang et al. 2007) which implies that the glyoxalase system is under dynamic regulation.

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## Conclusions and Perspectives

A sophisticated fine-tuned signaling and metabolic network is there under plant salinity response and tolerance. Significant discoveries of salinity-responsive genes, proteins, and metabolites in different cellular pathways important for salt stress response and tolerance have been made through previous morphological, physiological, genetic, and genomic analyses. But a large gap exists in the systematic understanding of the molecular processes and networks, as this understanding is still in the beginning. Modern highly sophisticated and state-of-the-art proteomics techniques have helped us in the acquisition of more detailed quantitative information on the temporal and spatial expression of proteins. Currently, overall 2,171 proteins have been identified in 34 plants, but the need of the hour is to fill the gaps present between the research and actual field conditions.

The dynamic nature of the proteins involved under salinity stress further provides invaluable information toward understanding of the underlying sophisticated cellular and molecular processes like photosynthesis, energy metabolism, ROS scavenging, ion/osmotic homeostasis,

signaling transduction, transcription and translational regulation, and cytoskeleton dynamics. However, a large gap still exists in our knowledge of transmembrane ion transport and cellular compartmentalization, sensors/receptors in signaling transduction, molecules in long distance signaling, and metabolites in energy supply, especially in case of cereals. Moreover, molecular interactions and pathway cross talks should have to be arranged, and future targets should be set on the basis of the gaps found during these talks. New state-of-the-art technologies like nanotechnology should be used in the understanding of molecular mechanisms. Through nanotechnology, enzyme biosensors (controlled-pore glass beads with optical transducer element, polyurethane foam with photothermal transducer element, ion-selective membrane with either potentiometric or amperometric transducer element, and screen-printed electrode with amperometric transducer element) should be used for the detection of very minute changes occurring during the quantitative analysis of the salt tolerance mechanisms. Also the advanced and sophisticated proteomics approaches and technologies like multidimensional protein fractionation, isobaric tags for relative and absolute quantitation (iTRAQ), label-free quantification mass spectrometry, and phosphoprotein and glycoprotein enrichment and tagging will definitely help us in discovering low-abundance proteins (e.g., transcriptional factors, kinases, channels, and transporters) and novel regulatory mechanisms (e.g., phosphorylation) in salt stress signaling and metabolism pathways. Integration of proteomics results with findings from other large scale sources will definitely facilitate in the establishment of molecular networks underlying salt stress response and tolerance in cereals. After integration of the knowledge collected, we will be able to predict the underlying molecular mechanisms of salinity tolerance. It will prove helpful toward the ultimate goal of improving plant salt tolerance for enhanced yield and bioenergy.

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