Advances in Understanding the Physiological and Molecular Responses of Sugar Beet to Salt Stress

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Soil salinity is a major environmental stress on crop growth and productivity. A better understanding of the molecular and physiological mechanisms underlying salt tolerance will facilitate efforts to improve crop performance under salinity. Sugar beet is considered to be a salt-tolerant crop, and it is therefore a good model for studying salt acclimation in crops. Recently, many determinants of salt tolerance and regulatory mechanisms have been studied by using physiological and 'omics approaches. This review provides an overview of recent research advances regarding sugar beet response and tolerance to salt stress. We summarize the physiological and molecular mechanisms involved, including maintenance of ion homeostasis, accumulation of osmotic-adjustment substances, and antioxidant regulation. We focus on progress in deciphering the mechanisms using 'omic technologies and describe the key candidate genes involved in sugar beet salt tolerance. Understanding the response and tolerance of sugar beet to salt stress will enable translational application to other crops and thus will have significant impacts on agricultural sustainability and global food security.

Keywords: sugar beet, salt stress, salt tolerance, proteomics, transcriptomics, metabolomics

INTRODUCTION

Soil salinity is a major environmental stress that affects agricultural production worldwide. Globally, about 960 million hectares of arable land are affected by excess salt levels (Munns and Tester, 2008; Deinlein et al., 2014; Jha et al., 2019). The adverse effects of an annual salinity increase of ~1-2% are exacerbated by global climate change, poor irrigation practice, and improper fertilizer utilization (Zhu, 2016). Furthermore, the human population is expected to reach 9.6 billion by 2050 (Shabala et al., 2015). The additional food required to feed this growing population has imposed great pressures on existing natural resources. Therefore, improving plant salt tolerance is useful for improving agricultural production and food security. Salinity causes ionic imbalance, osmotic stress, and secondary stresses, e.g., oxidative stress. These stresses have a substantial impact on crop life from seed germination to vegetative growth to reproduction and seed filling. Plant responses to salt stress include a decrease in leaf expansion, stomatal closure, inhibition of photosynthesis, and reduced biomass (Zhang and Shi, 2013). On the other hand, plants have evolved a large number of physiological and biochemical processes to adapt to salt stress. In general, the adaptive responses of

plants to salt stress can be grouped into three categories: osmotic stress tolerance, ion exclusion, and tissue tolerance to salinity (Yang and Guo, 2018a).

Sugar beet (*Beta vulgaris* L.) belongs to the order *Caryophyllales*, which lies at the basal taxa of core dicots. It is an important root crop in the world for sugar production, where its tap roots are used. The world production of sugar from sugar beet in 2018 was approximately 42 million metric tons, accounting for nearly 30% of the world sugar supply (FAO). In addition to its use in the sugar industry, it is a major source for animal feed and the production of bioethanol as renewable energy (Magaña et al., 2011). A wild ancestor of sugar beet is *Beta maritima* L. (sea beet), which grows naturally along the Atlantic coasts of Western Europe and the coasts of Mediterranean countries (Doney et al., 1999). To survive in these habitats, sea beet has developed structural and physiological strategies to regulate the distribution of salt and other solutes and to increase water content (Daoud et al., 2003). Many sugar beet cultivars inherited the salt tolerance trait from their ancestor and are considered salt-tolerant glycophytes. Sugar beet can tolerate up to 500 mM sodium chloride (NaCl) for seven days without losing viability (Yang et al., 2012). Moreover, it has been found that when the electrical conductivity (EC) of soil reached 7.0 dS m^{-1} (≈67 mM NaCl), the yield of sugar beet was not affected (Marschner, 1995). With the completion of the genome sequencing of sugar beet (Dohm et al., 2013), it is considered to be an excellent crop model for studying salt tolerance mechanisms.

Previous studies have mainly focused on the physiological responses of sugar beet to salt stress. Recently, more and more studies have focused on elucidating the molecular mechanisms of salt tolerance using different 'omics tools, such as RNA sequencing (Lv et al., 2018; Skorupa et al., 2019), proteomic analysis (Wakeel et al., 2011a; Yang et al., 2012; Yu et al., 2016), and metabolomic analysis (Hossain et al., 2017a). These large-scale studies have rapidly delivered new knowledge and important insights into the molecular processes of salt tolerance in sugar beet (**Table 1**). However, to date, there has been no critical review of the recent advances toward understanding the molecular and physiological mechanisms of sugar beet salt tolerance. This review article aims to fill this knowledge gap by providing an overview of recent progress made in the field of sugar beet salt tolerance, including its physiological and molecular mechanisms as revealed by 'omics technologies and the genes targeted for genetic improvement and molecular breeding for crop salt tolerance.

EFFECTS OF SALT STRESS

Although sugar beet is salt-tolerant compared to other crops, it is sensitive to salinity at the germination and early seedling stages (Kaffka and Hembree, 2004; Sadughi et al., 2015). Water uptake and availability is essential for seed germination and early seedling growth. Salt stress causes a significant reduction in water availability, decreases the mobilization of stored reserves, and affects the structural organization of proteins, leading to poor germination (Sadughi et al., 2015). However, the usual long lag time of sugar beet seeds in salinized soil may initiate seed priming (AboKassem, 2007; Sadeghian and Yavari, 2010), enabling germination at a salinity level of 12 dS m^{-1} (≈115 mM

TABLE 1 | Major 'omic studies of salt stress tolerance in sugar beet using different technological platforms.

Tissue/Species	Salt treatment	Technique	Key findings	Reference
Leaves and roots/*Bv*M14	0/400 mM NaCl	SSH	Identified differentially expressed genes in *Bv*M14 under salt stress	Ma et al., 2017
Leaves and roots/*Bv*M14	0/200/400 mM NaCl	RNA-SEQ	Identified differentially expressed genes in *Bv*M14 under salt stress	Lv et al., 2018
Leaves/*B. maritime*	0/150/300 mM NaCl	RNA-SEQ	Investigated transcriptome response to acute salt stress imposed to excised leaves of sea beet	Skorupa et al., 2016
Leaves/*B. maritime* and *B. vulgaris*	0/300 mM NaCl	RNA-SEQ	Revealed alterations in beet leaf transcriptome during acclimation to stress and response to shock, and identified salinity-related and genotype-specific traits in the patterns of gene expression in leaves of sea beet and sugar beet.	Skorupa et al., 2019
Leaves and roots/*Bv*M14	0/200/400 mM NaCl	2D-DIGE/iTRAQ	Analyzed salt-responsive proteins in *Bv*M14 plants under salt stress	Yang et al., 2012; Yang et al., 2013
Leaves and roots/*B. vulgaris* (T510, salt-tolerant) and *B. vulgaris* (S210, salt- sensitive)	0/280 mM NaCl	iTRAQ	Identified differentially changed proteins between the sensitive and tolerant cultivars of sugar beet, and provided a list of potential markers for the further engineering of salt tolerance in crops	Wang et al., 2019
Leaves/*Bv*M14	0/200/400 mM NaCl	iTRAQ	Analyzed the *Bv*M14 membrane proteome under salt stress	Li et al., 2015
Leaves/*Bv*M14	0/200/400 mM NaCl	Label-free quantitative proteomics	Profiled changes in the *Bv*M14 phosphoproteome induced by salt stress	Yu et al., 2016
Leaves/*B. vulgaris*	0/300 mM NaCl	GC-MS	Investigated metabolic adaptations of sugar beet to salt stress through GC-MS of whole leaf tissues and chloroplasts	Hossain et al., 2017a

SSH, suppression subtractive hybridization; RNA-SEQ, RNA Sequencing; iTRAQ, isobaric tag for relative and absolute quantification; 2D-DIGE, two-dimensional difference gel electrophoresis.

NaCl), which does not significantly affect seed germination compared to control conditions (Jafarzadeh and Aliasgharzad, 2007; Khayamim et al., 2014). When the salinity level reached 16 dS m⁻¹ (≈150 mM NaCl) or a combination of different salts compositions (Mg₂SO₄/NaCl/Na₂SO₄/CaCl₂) were imposed, sugar beet seed germination was found to be significantly inhibited (Jafarzadeh and Aliasgharzad, 2007). Presumably, decreased osmotic potential caused by high salt concentration inhibits water imbibition in seeds. The establishment of sugar beet seedlings is more susceptible to salinity inhibition than is seed germination. For example, the root length is significantly decreased at an EC of 4 dS m⁻¹ (≈38 mM NaCl) (Jafarzadeh and Aliasgharzad, 2007). It was also reported that the hypocotyl length and number of normal seedlings were negatively correlated with the increase of salt level. Therefore, long roots and hypocotyls and a low percentage of abnormal seedlings (as defined by the International Seed Testing Association, 1985) may be used as indexes for identifying salt-tolerant genotypes of sugar beet (Khayamim et al., 2014).

Chlorophyll is the major pigment in plant photosynthesis and is responsible for absorbing and transforming light energy. Chlorophyll content is thus an important physiological indicator of plant salt-stress damage. The content of chlorophyll in sugar beet was decreased by 38.4% at 280 mM NaCl (EC≈33 dS m⁻¹) but did not change under mild salinity (EC = 5.5 dS m⁻¹ ≈55 mM NaCl.) (Hajiboland et al., 2009; Wang et al., 2017). In addition, the net photosynthesis rate and stomatal conductance showed similar change trends to those of the chlorophyll level under the above two salt-stress conditions. Rubisco, which is directly involved in CO₂ fixation, is a determining factor for carbohydrate accumulation in plants. Its activity was obviously decreased under salt stress in comparison with control (Hossain et al., 2017b; Wang et al., 2017). These adverse effects eventually lead to decreases in the leaf area, growth, and root yield of sugar beet.

Salt stress also affects the uptake and accumulation of mineral nutrients in sugar beet. For example, high salinity increases the phosphate content of sugar beet in a dose-dependent manner. Excessive accumulation of phosphorus may lead to phosphorus poisoning, growth retardation, and necrosis in plants (Zhou et al., 2008). Nitrate reductase (NR) is a key enzyme in plant nitrogen acquisition and is responsible for the synthesis of nitric oxide (NO), a key signaling molecule in plant cells (Chamizo-Ampudia et al., 2017). NR activity was found to be significantly inhibited by NaCl in young and old leaves of sugar beet. The degree of decrease in the enzyme activity was greater with increasing salt concentration (Ghoulam et al., 2002). The reduction of NR activity was mainly due to the excess accumulation of salt in the cytoplasm. However, direct links between salt and NR regulation are not known. Another report showed that salinity induced lower nitrogen assimilation in sugar beet at the end of its growth season compared to earlier in the season (Hajiboland et al., 2009). Although this situation caused sugar beet to have a lower dry matter and shoot mass, the weight of the storage root and the sugar content increased significantly because of a change in the partitioning of organic materials in favor of the roots.

PRIMARY MECHANISMS OF SALT TOLERANCE IN SUGAR BEET

Accumulation of Osmotic Adjustment Substances

Osmotic stress is the primary form of stress suffered by plants when subjected to salt stress. Osmotic adjustment is vital for the alleviation of the osmotic imbalances caused by salt stress and for maintaining cell turgor (Liang et al., 2018). It involves cellular solute accumulation in response to a decrease in the water potential of the environment. Although sugar beet is sensitive to salinity at seed germination and early seedling stage (see the previous section), established plants exhibit a high osmotic adjustment capacity, as reflected by the accumulation of organic and inorganic osmolytes under salt stress (Katerji et al., 1997; Wu et al., 2015a). Several studies have found that glycinebetaine (GB), choline, free amino acids, and proline accumulated in sugar beet leaves with increasing NaCl concentrations in the growth medium (Ghoulam et al., 2002; Yamada et al., 2009; Wu et al., 2013a). High levels of GB (>20 mmol g FW⁻¹) in young leaves of sugar beet were detected under normal growth conditions (Yamada et al., 2009). In contrast, the contents of GB in old leaves, cotyledons, hypocotyls, and roots were low. GB is primarily synthesized in old leaves and is translocated into young leaves (Yamada et al., 2009). As a result, high accumulation of GB was observed in the new leaves of sugar beet, especially under salt-stress conditions (Russell et al., 1998; Yamada et al., 2009). GB clearly plays a key role in the osmotic adjustment of sugar beet (Waditee et al., 2007). GB is synthesized via a two-step reaction catalyzed by choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH) (Chen and Murata, 2002). CMO catalyzes the first step, which is the rate-limiting step in GB synthesis (Hibino et al., 2002). Antisense BvCMO transgenic sugar beets with suppressed levels of BvCMO protein exhibited decreased GB synthesis and became more susceptible to salt stress compared to wild-type plants (Yamada et al., 2015). On the other hand, transplastomic tobacco plants over-expressing the BvCMO gene accumulated GB and exhibited increased tolerance to salt stress (Zhang et al., 2008). Thus, BvCMO has the potential to be used in genetic engineering to improve plant salt stress tolerance. GB was found to be transported by proline transporters using a transmembrane proton gradient (Yamada et al., 2011). Whether the transport and distribution of GB in different plant tissues affects plant salt tolerance needs further investigation.

Proline is another important osmolyte in plant cells, and its cellular levels can be used as a physiological index of plant salt stress tolerance in many species (Per et al., 2017; De la Torre-González et al., 2018). However, the importance of proline accumulation in sugar beet under salt stress for osmotic adjustment is still under debate. It is noteworthy that shoot proline concentrations were significantly higher in salt-tolerant cultivars than in salt-sensitive cultivars under control or salt-stress conditions (Ghoulam et al., 2002; Wu et al., 2013a). It was speculated that high proline contents in the salt-tolerant sugar beet genotypes could be induced by cellular demand for osmotic adjustment and membrane stabilization. However, from a quantitative point of

view, the contribution to osmotic adjustment by the accumulated proline appeared to be small compared to GB. Furthermore, inorganic salt ions may play a more important role than proline in osmotic adjustment. It was reported that sugar beet seedlings accumulate high levels of ions such as Na^+, K^+, and Cl^- in their shoots, which are involved in effective osmotic adjustment under salt-stress conditions (Wu et al., 2015b).

Redox Regulation of Salt Tolerance

Salt stress induces the accumulation of cellular reactive oxygen species (ROS), such as superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot OH$), that are generated by plant photosynthetic and respiratory electron transport systems, xanthine oxidases, and NADPH oxidases. Usually, cellular ROS levels are regulated by both non-enzymatic antioxidants and enzymatic antioxidants (Apel and Hirt, 2004; Baxter et al., 2014; Ben Rejeb et al., 2015). Interestingly, H_2O_2 levels in sugar beet were found to be lower under long-term salinity than under control conditions (Hossain et al., 2017b). This result is quite different from the responses of other crop species, which exhibit salt-stress-induced ROS accumulation (Mittler et al., 2004). It is speculated that sugar beet is efficient in adjusting the cellular redox environment under salinity. The expression of *superoxide dismutases (SOD)* (e.g., *Cu-Zn-SOD*, *Mn-SOD*, and *Fe-SOD*) and *peroxiredoxins (Prx)* were increased under salt stress (Hossain et al., 2017b). The induced expression of these antioxidant-related genes helps to remove accumulations of ROS. In the meantime, transcription of *respiratory burst oxidase homolog* (*RBOH*) isoforms, the major ROS-generating *NADPH oxidases*, were significantly suppressed under salinity. These mechanisms of maintaining low ROS accumulation help to mitigate oxidative stress and facilitate normal cellular metabolism for the growth and development of sugar beet under salt stress.

Changes in antioxidant enzymes involved in salt stress tolerance have also been investigated in the cultivated sugar beet *B. vulgaris* and its wild salt-tolerant relative *B. maritime*. The activities of SOD, peroxidase (POX), ascorbate peroxidase (APX), catalase (CAT), and glutathione reductase (GR) in *B. maritime* were obviously higher than in *B. vulgaris* (Bor et al., 2003). APX is one of the major members in the anti-oxidation system that scavenges the excess H_2O_2 caused by salt stresses (Guan et al., 2015). APX can convert H_2O_2 into H_2O with ascorbic acid (ASA) as the electron donor. Interestingly, the expression of *BvAPX* was up-regulated under salt stress in the leaves of both *B. maritime* and *B. vulgaris*. However, a much longer duration of salt stress was required to induce *APX* gene expression in the salt-tolerant *B. maritime* compared to in the salt-sensitive varieties (Dunajska-Ordak et al., 2014). The highly efficient osmotic regulation in *B. maritime* may account for the delayed induction of *APX* expression. Peroxidase (POX) is another well-known antioxidant enzyme that protects plant cells from oxidative damage, and its enzymatic activity was induced in the roots of sugar beet under stress conditions (Pradedova et al., 2014). Recently, it was reported that the level of *POX* gene transcription under salt stress was related to the elevated levels of acetylation in H3K9 and H3K27 sites in sugar beet (Yolcu et al.,

2016). In *B. vulgaris* and *B. maritime,* the acetylation patterns were significantly different. Unlike the cultivated *B. vulgaris*, the main acetylation site of the salt-tolerant wild species is H3K9. These studies indicate that the antioxidant system plays a key role in determining sugar beet salt tolerance, and epigenetics appears to regulate the antioxidant system at the transcriptional level.

Maintaining Ion Homeostasis

When plants are exposed to a saline environment, Na^+ can enter cells through non-selective cation channels and K^+ transporters (Ward et al., 2003). Thus, maintaining ion homeostasis is imperative for plants to adapt to salt stress (Nadeem et al., 2019). Usually, plants eliminate excessive Na^+ from the cytosol *via* the plasma membrane or tonoplast Na^+/H^+ antiporters (NHX) to maintain an optimal cytosolic Na^+ level (Zhu, 2003; Olías et al., 2009). These Na^+/H^+ antiporters use the electrochemical gradient of protons across the tonoplast or plasma membrane to move Na^+ into the vacuole or outside the cell, respectively (Blumwald, 2000). It has been suggested that the activity of vacuolar Na^+/H^+ antiporters is significantly different in salt-tolerant versus salt-sensitive plants. Compared with salt-sensitive plants, *NHX* transcription in salt-tolerant plants is much more strongly induced (Yokoi et al., 2002; Gong et al., 2010). Furthermore, tonoplasts of salt-tolerant sugar beet exhibited high tonoplast NHX activity, which was directly related to the salt stress tolerance of *B. vulgaris* cell cultures (Blumwald and Poole, 1987; Wu et al., 2019). The transcription of *BvNHX1* was significantly increased under salt stress. This pattern of increase was consistent with elevated BvNHX1 protein and vacuolar NHX activity (Xia et al., 2002). Another study showed that the sugar beet *BvNHX1* gene was modulated by MYB transcription factor(s), which were responsible for activating its expression upon salt exposure (Adler et al., 2010). Interestingly, overexpression of tonoplast *NHXs* in sugar beet was shown to improve salt stress tolerance (Yang et al., 2005; Liu et al., 2010). The transgenic sugar beet accumulated high levels of potassium and low levels of salt in the roots. Furthermore, the transgenic sugar beet exhibited higher soluble sugar content and yield in storage roots under saline conditions than the wild type (Liu et al., 2015). Simultaneously with the activation of vacuolar *BvNHX* under salt stress, the transcription of vacuolar H^+ pump *V-H⁺-ATPase* was also enhanced (Kirsch et al., 1996). This coordinated regulation of both *NHX* and *V-H⁺-ATPase* in sugar beet constitutes an efficient mechanism underlying vacuolar salt sequestration and salt tolerance in sugar beet (Kirsch et al., 1996).

The restriction of K^+-efflux is another important mechanism underlying the salt tolerance of sugar beet. Plasma membrane (PM) H^+-ATPase was found to be involved in restricting K^+ efflux under salt-stress conditions (Sun et al., 2010; Shabala et al., 2016). A previous study compared the effect of salt stress on PM H^+-ATPase activity in salt-sensitive maize and salt-tolerant sugar beet (Wakeel et al., 2011b). Although high concentrations of salt can inhibit PM H^+-ATPase activity, the activity in the salt-tolerant sugar beet was less affected than that in maize. At low concentrations of salt, the PM H^+-ATPase activity in sugar beet was not affected, while an obvious decrease of activity was

detected in maize. These results indicate that PM H⁺-ATPase in sugar beet is relatively stable under salt stress and can maintain a high level of cellular K^+. In addition, apoplastic pH, which is determined by the H^+-ATPases, was not affected in sugar beet under salt stress (Kirsch et al., 1996; Wakeel et al., 2010). According to the acid-growth theory, lower apoplastic pH allows extension growth by increasing cell wall extensibility. Therefore, the relatively stable PM H^+-ATPase under salt stress ensures an acid-growth condition for sugar beet.

'OMICS TECHNOLOGIES FOR DISCOVERING THE GENES, PROTEINS AND METABOLITES INVOLVED IN SALT TOLERANCE
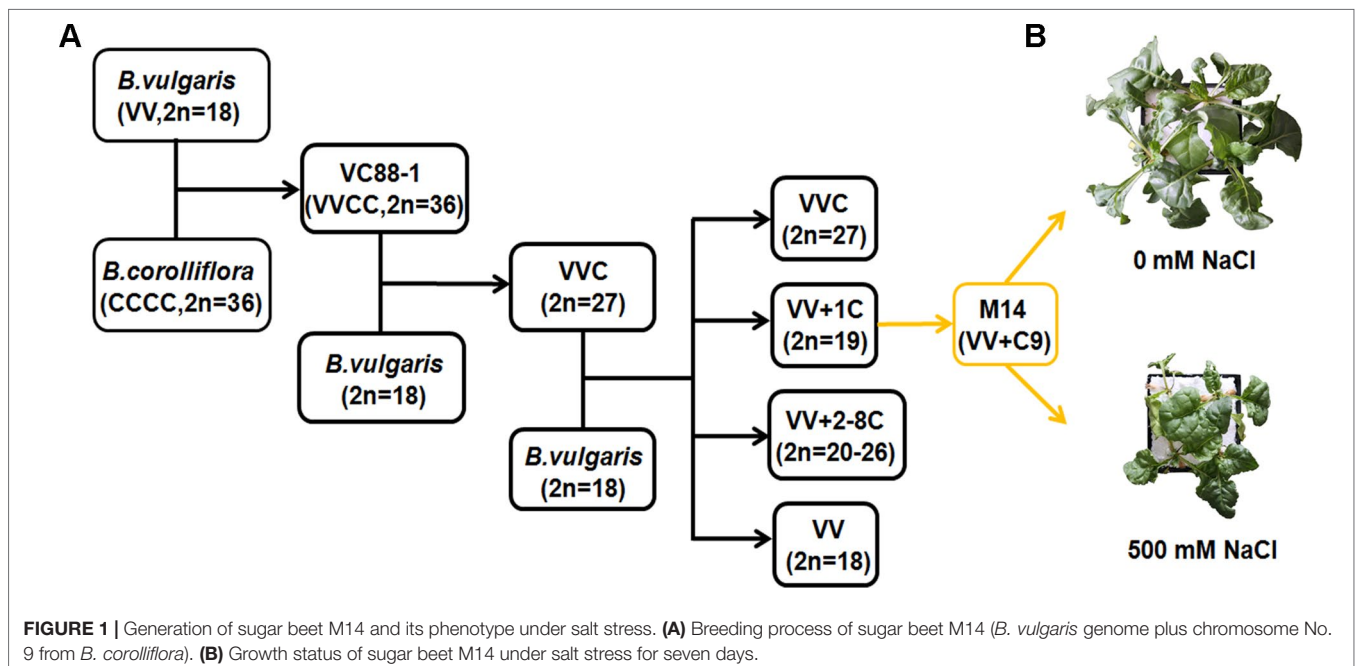
To date, many studies have focused on utilizing 'omics tools to explore salt-tolerance mechanisms in plants. The 'omics tools include genomics, transcriptomics, proteomics, and metabolomics, which allow large-scale discovery of candidate genes, proteins, and metabolites involved in plant salt stress tolerance (Sun et al., 2017; Lei et al., 2018). Recently, several important genes, proteins and metabolites related to salt tolerance in sugar beet have been reported (**Table 1**). Here we describe how the 'omics results help to improve understanding of the salt-tolerance mechanisms in sugar beet.

Transcriptomic Study of Salt Tolerance in Sugar Beet

In the early days, the polymerase chain reaction (PCR)-based suppression subtractive hybridization (SSH) method was adopted to compare gene expression patterns between 'tester' and 'driver'

populations (Wang et al., 2005). A number of differentially expressed genes related to salt-stress tolerance were identified in different plant species by the SSH technique (Baldwin and Dombrowski, 2006; Ouyang et al., 2006). In our previous work, a single chromosome from *B. corolliflora* was introduced into the cultivated *B. vulgaris* through the traditional crossing of distant species. One of the hybrid lines, *Bv*M14, containing chromosome 9 of *B. corolliflora* was obtained (**Figure 1**). It showed characteristics of apomixis and tolerance to salt stress (Li et al., 2010). *Bv*M14 tolerated up to 500 mM NaCl treatment (**Figure 1**). The SSH method was applied to explore changes in the transcriptional profiles of the *Bv*M14 plants under salinity (Ma et al., 2017). Tester and driver cDNAs were synthesized from *Bv*M14 root and leaf mRNA extracted from control and salt-stress plants. A total of 36 differentially expressed genes were identified and annotated in *Bv*M14 roots and leaves under salt stress. Most of the genes were involved in metabolism and photosynthesis. For example, one of the differentially expressed genes, *S-adenosylmethionine synthetase 2* (*Bv*M14-SAMS2), is an important enzyme in the synthesis of S-adenosylmethionine (SAM), a precursor of polyamines. It was suggested that polyamines may be involved in determining plant responses to abiotic or biotic stresses. As expected, transgenic *Arabidopsis* plants overexpressing *Bv*M14-SAMS2 exhibited strong salt and H_2O_2 tolerance compared to the wild type (Ma et al., 2017). Another salt-responsive gene, *Bv*M14-cystatin, identified by the SSH method, was also isolated from sugar beet and overexpressed in *Arabidopsis*. The transgenic plants showed enhanced salt tolerance (Wang et al., 2012). Taken together, these studies showed that the SSH technique was useful for identifying key genes involved in sugar beet salt tolerance in the early days.

Recently, high-throughput sequencing tools developed to monitor gene expression patterns have made it possible to

FIGURE 1 | Generation of sugar beet M14 and its phenotype under salt stress. **(A)** Breeding process of sugar beet M14 (*B. vulgaris* genome plus chromosome No. 9 from *B. corolliflora*). **(B)** Growth status of sugar beet M14 under salt stress for seven days.

systemically explore how plants cope with salt stress (Sun et al., 2013; Bushman et al., 2016). In order to determine a broad spectrum of genes involved in *Bv*M14 salt tolerance, comparative transcriptomics analysis was performed to identify differentially expressed genes in the leaf and root samples of *Bv*M14 seedlings at 0, 200 and 400 mM NaCl conditions (Lv et al., 2018). GO and KEGG enrichment analyses found that a large proportion of the differentially expressed genes were concentrated in redox balance, signal transduction, and protein phosphorylation. In addition, the differentially expressed genes of *Bv*M14 under salt stress were very different in the leaves and in the roots. This result indicates that the *Bv*M14 roots and leaves have different adaptation mechanisms for coping with salt stress. Moreover, the genes involved in the ROS scavenging system, such as *APX, SOD, thioredoxin (TRX), glutathione S-transferase (GST), monodehydroascorbate reductase (MDAR)*, and *glycolate oxidase (GOX)*, showed significant differences in sugar beet under salt stress compared to control. This result indicates that the plant antioxidant system plays an important role in regulating sugar beet salt tolerance.

As sea beet (*B. maritima*) displays elevated salt-tolerance compared to the cultivated beet (*B. vulgaris*), a study also investigated the transcriptomic responses to acute salt stress imposed to excised leaves of sea beet. Differentially expressed genes involved in osmoprotection, molecular chaperoning, and redox protein synthesis were identified and may play a key role in determining salt tolerance in sea beets (Skorupa et al., 2016). Recently, another study using RNAseq was published that explored transcriptional patterns related to salt responses in sugar beet (*B. vulgaris*) and its wild ancestor sea beet (Skorupa et al., 2019). Two kinds of salt treatment strategies were applied: either a gradual increase in salt concentration (salt-stress) or sudden exposure to salinity (salt-shock). Interestingly, sugar beet exhibited more significant transcriptomic changes to maintain homeostasis than sea beet. In addition, salt shock induced greater transcriptomic changes than salt stress, and salt shock led to a larger number of up-regulated genes compared to salt stress. Moreover, this study also confirmed that sugar beet inherited salt-tolerance traits from sea beet and that bHLH transcription factors are candidate regulators of salt-stress responses in sugar beet (Skorupa et al., 2019).

Proteomic Study of Salt Tolerance in Sugar Beet

Although some of the salt-responsive genes have been identified in sugar beet using high-throughput RNA sequencing, transcriptomic data does not often correlate with the results of proteomic data due to posttranscriptional, translational, and posttranslational regulations (Chen and Harmon, 2006; Li et al., 2015; Walley et al., 2016). Therefore, it is imperative to employ proteomics to investigate global protein level changes under salt stress. Previously, two sugar beet cultivars with contrasting salt tolerance were selected to compare their proteomes' response to salt using an isobaric tag for a relative and absolute quantification (iTRAQ)-based proteomic approach (Wang et al., 2019). This study indicated that salt-sensitive and -tolerant sugar beet cultivars exhibited different changes in proteomic profiles under salt stress. Several proteins involved in protein modification, the tricarboxylic acid cycle, cell wall synthesis,

and reactive oxygen species scavenging showed differential changes between the sensitive and tolerant cultivars, indicating that these pathways may participate in the salt tolerance of sugar beet. Some potential markers for further engineering of salt tolerance in sugar beet have been identified, such as late embryogenesis abundant (LEA) proteins, abscisic acid-stress ripening protein 1 (ASR1), and S-adenosylhomocysteine synthase. Furthermore, salt-responsive characteristics of the salt-tolerant sugar beet *Bv*M14 were studied under 0, 200, and 400 mM NaCl conditions using two-dimensional difference gel electrophoresis (2D-DIGE) and gel-free iTRAQ approaches (Yang et al., 2012; Yang et al., 2013). Differentially expressed proteins involved in photosynthesis, respiration, the antioxidant system, methionine metabolism, and GB synthesis were all increased in the roots and leaves of *Bv*M14. The results indicated that enhancement of photosynthesis and methionine metabolism, accumulation of compatible organic solutes and antioxidative enzymes, and increase in ion uptake/exclusion were the main regulatory mechanisms underlying salt tolerance in *Bv*M14. One of the differentially expressed proteins, *Bv*M14-glyoxalase I, was found to exhibit a two-fold increase in leaves in response to salt treatment. *Bv*M14-glyoxalase I was ubiquitously expressed in different tissues of *Bv*M14 and displayed increased levels under salt, mannitol, and oxidative stresses (Wu et al., 2013b). To investigate the functions of *Bv*M14-glyoxalase I, it was constitutively expressed in *Nicotiana tabacum*. The transgenic plants showed marked tolerance to methylglyoxal, salt, mannitol, and H_2O_2. Another differential protein in *Bv*M14, S-adenosylmethionine decarboxylase (*Bv*M14-SAMDC), is a key rate-limiting enzyme involved in plant polyamine synthesis. Recently, the gene encoding the *Bv*M14-SAMDC protein was cloned from *Bv*M14 leaves (Ji et al., 2019). When *Bv*M14-SAMDC was overexpressed, increased levels of spermidine (Spd) and spermin (Spm) and high activities of antioxidant enzymes were observed compared to in the wild type. Interestingly, expression levels of *RbohD* and *RbohF*, which play a role in ROS production, were significantly decreased in the transgenic plants (Ji et al., 2019). This study indicates that biosynthesis of Spm and Spd contributes to sugar beet salt stress tolerance through enhanced antioxidant activities and decreased ROS generation.
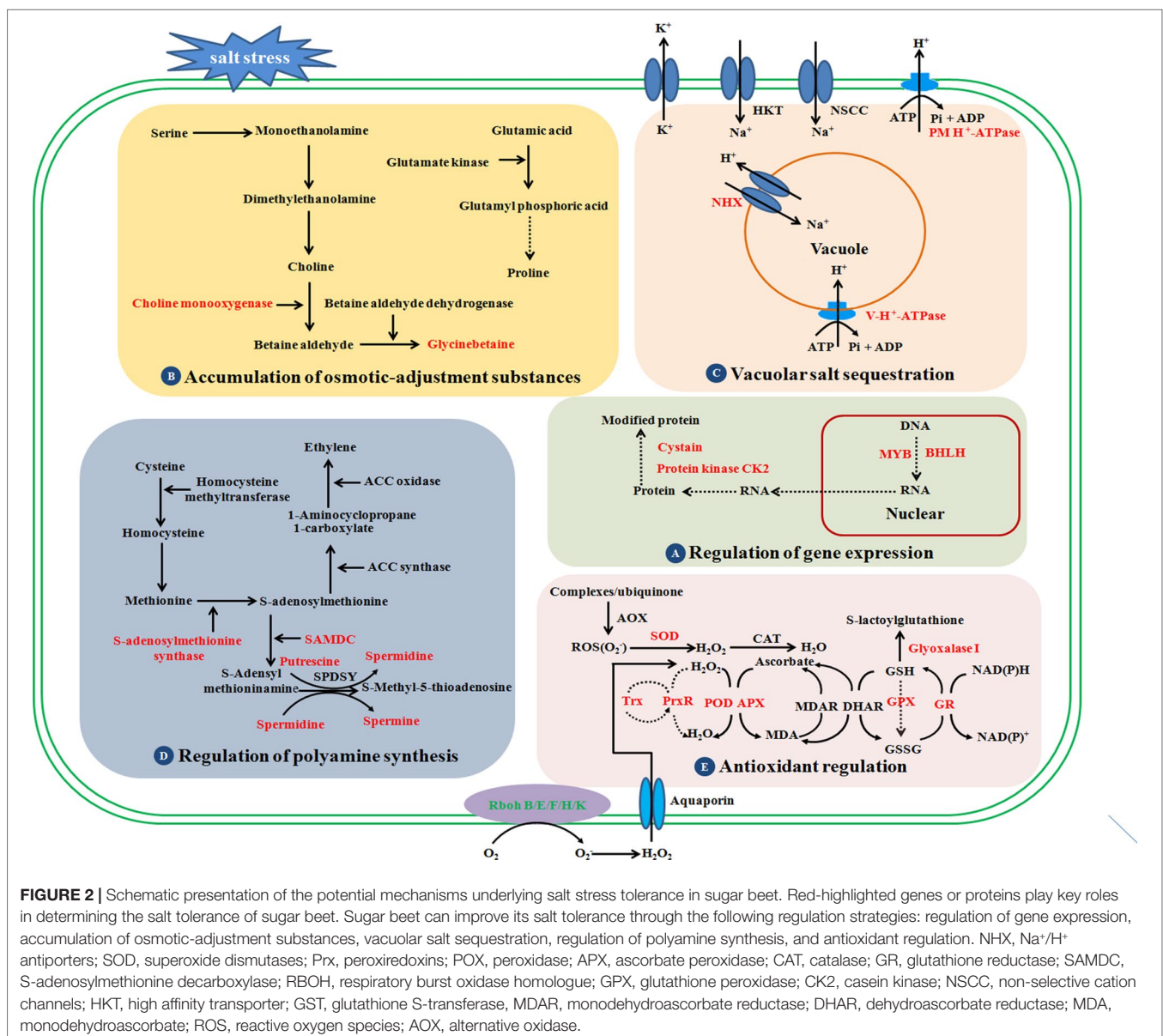
Plant cells have extensive membrane systems that play a key role in regulating responses and adaptation to salt stress. To understand the essential functions of membrane systems, an iTRAQ-based comparative proteomic study was conducted using microsomes of sugar beet *Bv*M14 plants under control and salt-stress conditions (Li et al., 2015). This study revealed that plasma membrane ATPase 11 and vacuolar ATPase subunit H were increased in response to salt stress. These proteins were involved in generating a proton gradient for ion transport across the plasma membrane and vacuolar membrane, respectively. Increasing the levels and activity of the ATPases may be an effective strategy for Na^+ sequestration and osmotic adjustment under salt stress. Protein phosphorylation is one of the most widespread post-translational regulations in plant cell signaling under salt stress (Hubbard and Cohen, 1993; Tanou et al., 2012). Changes in the phosphoproteome of *Bv*M14 plants under short-term salt stress were analyzed using label-free quantitative proteomics (Yu et al., 2016). Several key kinases were found to exhibit significant changes under salt stress, including mitogen-activated

protein kinases (MAPKs), 14-3-3s, receptor kinases, and calcium-dependent protein kinases (CDPKs). In addition, the phosphorylation of peroxiredoxin and several other proteins was a novel and intriguing discovery. Furthermore, sugar beet casein kinase 2 (CK2) was induced by salt stress and has been proved critical to salt tolerance in *Saccharomyces cerevisiae* (Kanhonou et al., 2001). With the exception of phosphorylation, other post-translational modifications have rarely been studied in sugar beet and should be an exciting subject for future research.

Metabolomic Study of Salt Tolerance in Sugar Beet

Global metabolic changes can reflect protein activities and physiological responses to different environmental stresses in plants. Therefore, metabolomics is an important functional genomics tool that complements genomics and proteomics (Clément et al., 2018; Ghatak et al., 2018). However, metabolomic analysis of sugar beet stress responses has been rare. Using gas chromatography-mass spectrometry (GC-MS), Hossain et al. (2017a) analyzed metabolic changes in the whole leaves and chloroplasts of sugar beet in response to salt stress. Metabolites involved in the Calvin-Benson cycle, glycolysis, and the citric acid cycle exhibited significant decreases in leaves under salt stress. In contrast, the levels of glycolate and serine increased significantly. This result indicates that photorespiratory metabolism is enhanced in the salt-stressed sugar beet. In addition, arabinose, glycolate, inositol, malate, mannitol, and putrescin were found to accumulate in both chloroplasts and extra-chloroplastic space to help maintain the chloroplast biochemical processes through

FIGURE 2 | Schematic presentation of the potential mechanisms underlying salt stress tolerance in sugar beet. Red-highlighted genes or proteins play key roles in determining the salt tolerance of sugar beet. Sugar beet can improve its salt tolerance through the following regulation strategies: regulation of gene expression, accumulation of osmotic-adjustment substances, vacuolar salt sequestration, regulation of polyamine synthesis, and antioxidant regulation. NHX, Na$^+$/H$^+$ antiporters; SOD, superoxide dismutases; Prx, peroxiredoxins; POX, peroxidase; APX, ascorbate peroxidase; CAT, catalase; GR, glutathione reductase; SAMDC, S-adenosylmethionine decarboxylase; RBOH, respiratory burst oxidase homologue; GPX, glutathione peroxidase; CK2, casein kinase; NSCC, non-selective cation channels; HKT, high affinity transporter; GST, glutathione S-transferase, MDAR, monodehydroascorbate reductase; DHAR, dehydroascorbate reductase; MDA, monodehydroascorbate; ROS, reactive oxygen species; AOX, alternative oxidase.

TABLE 2 | Candidate genes for improving salt tolerance in sugar beet.

Candidate genes	Species	Function	Reference
BvCMO	*Beta vulgaris*	Synthesis of glycinebetaine	Zhang et al., 2008
BvSOD	*B. vulgaris*	Removing accumulation of ROS	Hossain et al., 2017b; Bor et al., 2003
BvPrx	*B. vulgaris*	Removing accumulation of ROS	Hossain et al., 2017b; Bor et al., 2003
BvCAT	*B. maritime* and *B. vulgaris*	Removing accumulation of ROS	Bor et al., 2003
BvGR	*B. maritime* and *B. vulgaris*	Removing accumulation of ROS	Bor et al., 2003
BvAPX	*B. maritime* and *B. vulgaris*	Removing accumulation of ROS	Dunajska-Ordak et al., 2014
BvPOX	*B. maritime* and *B. vulgaris*	Removing accumulation of ROS	Yolcu et al., 2016
BvNHX1	*B. vulgaris*	Vacuolar sequestration of sodium	Adler et al., 2010
MYB transcription factor	*B. vulgaris*	Regulating expression of NHX1	Adler et al., 2010
bHLH transcription factor	*B. maritime* and *B. vulgaris*	Regulating salt stress response	Skorupa et al., 2019
PM H⁺-ATPase	*B. vulgaris*	Maintaining ion homeostasis	Kirsch et al., 1996; Wakeel et al., 2010
BvM14-SAMS2	*Bv*M14	Synthesis of polyamines	Ma et al., 2017
BvM14-SAMDC	*Bv*M14	Synthesis of polyamines	Ji et al., 2019
BvM14-glyoxalase I	*Bv*M14	Detoxifying methylglyoxal	Wu et al., 2013b
BvM14-cystatin	*Bv*M14	Increasing salt tolerance	Wang et al., 2012
BvCK2	*B. vulgaris*	Signal transduction and stress response	Kanhonou et al., 2001

NHX, Na^+/H^+ antiporters; SOD, superoxide dismutases; Prx, peroxiredoxins; POX, peroxidase; APX, ascorbate peroxidase; CAT, catalase; GR, glutathione reductase; SAMDC, S-adenosylmethionine decarboxylase; SAMS, S-adenosylmethionine synthetase; GPX, glutathione peroxidase; CK2, casein kinase; ROS, reactive oxygen species; AOX, alternative oxidase; CMO, choline monooxygenase.

osmotic adjustment. This study also found high levels of the metabolite polyamine putrescine in the chloroplasts, which may play an important role in the acclimation of sugar beet to high salinity stress. The result is consistent with the findings at the transcriptomic level (Ma et al., 2017) and proteomic level (Ji et al., 2019). Since GC-MS profiles primarily central metabolites, liquid chromatography-MS-based untargeted metabolomics approaches (Geng et al., 2016; Geng et al., 2017) may greatly enhance the coverage of the sugar beet metabolome.

DISCUSSION

Soil salinization is increasingly problematic and has become a prime concern for global crop production and food security. During evolution, sugar beet has developed various adaptations to combat salt stress, such as osmotic adjustment and osmoregulation, activation of antioxidant defense systems, control of ROS accumulation, and maintenance of ion homeostasis (**Figure 2**). Although sugar beet is more salt-tolerant than other crops, high concentrations of salt significantly affect its yield and quality. There is an immense need to create new sugar beet varieties with stable and high yield in highly saline environments. In the past two decades, several salt tolerance determinants and signaling pathways have been identified in sugar beet, and some of the key candidate genes have been screened for improving sugar beet salt tolerance, such as *CMO*, which is involved in the biosynthesis of GB (**Table 2**, **Figure 2**). Sugar beet salt tolerance is a quantitative trait that is controlled by multiple genes. However, there are few reports on molecular markers and quantitative trait loci (QTLs) associated with salt tolerance. Studies of the salt-overly-sensitive (SOS) pathway and MAPK-related cascades have provided important information about Na^+ efflux, osmotic, and oxidative stress signaling in the reference plant *Arabidopsis thaliana* (Yang and Guo, 2018a; Yang and Guo, 2018b). Whether these signal transduction pathways

play a key role in determining salt tolerance in sugar beet still needs further study. Moreover, many determinants involved in sugar beet tolerance have not been studied in sufficient detail, such as salt-stress signal perception and crosstalk, developmental regulation, and the most important osmotic regulator(s) for salt-tolerance, etc. To date, transcriptomics and proteomics have facilitated our understanding of the nodes and edges in the salt-stress molecular networks, but metabolomics, epigenetics, and multi-omics integrative studies are still lacking. In addition, studies using sugar beet genetic resources (e.g., *Bv*M14) and functional characterization of promising molecular markers for salt-stress tolerance will greatly advance this exciting field of research, which has great potential in agricultural applications.

AUTHOR CONTRIBUTIONS

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