10

Mechanisms of Sugar Beet Response to Biotic and Abiotic Stresses

Bing Yu, Mengdi Chen, Inga Grin, and Chunquan Ma

Abstract

Sugar beet is used not only in the sugar production, but also in a wide range of industries including the production of bioethanol as a source of renewable energy, extraction of pectin and production of molasses. The red beetroot has attracted much attention as health-promoting and disease-preventing functional food. The negative effects of environmental stresses, including abiotic and biotic ones, significantly decrease the cash crop sugar beet productivity. In this paper, we outline the mechanisms of sugar beet response to biotic and abiotic stresses at the levels of physiological change, the genes' functions, transcription and translation. Regarding the physiological changes, most research has been carried out on salt and drought stress. The functions of genes from sugar beet in response to salt, cold and heavy metal stresses were mainly investigated by transgenic technologies. At the transcriptional level, the transcriptome analysis of sugar beet in response to salt, cold and biotic stresses were conducted by RNA-Seq or SSH methods. At the translational level, more than 800 differentially expressed proteins in response to salt, K^+/Na^+ ratio, iron deficiency and resupply and heavy metal (zinc) stress were identified by quantitative proteomics techniques. Understanding how sugar beet respond and tolerate biotic and abiotic stresses is important for boosting sugar beet productivity under these challenging conditions. In order to minimize the negative impact of these stresses, studying how the sugar beet has evolved stress coping mechanisms will provide new insights and lead to novel strategies for improving the breeding of stress-resistant sugar beet and other crops.

Keywords

Sugar beet · Biotic and abiotic stresses · Physiological change · Gene function · Transcriptomics · Proteomics

Co-Correspondent Authors Inga Grin and Chunquan Ma.

B. Yu (✉) · M. Chen · C. Ma (✉)
Engineering Research Center of Agricultural Microbiology Technology, Ministry of Education, Heilongjiang University, Harbin, China

Key Laboratory of Molecular Biology, College of Heilongjiang Province, School of Life Sciences, Heilongjiang University, Harbin, China

I. Grin (✉)
SB RAS Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia

Novosibirsk State University, Novosibirsk, Russia
e-mail: grin@niboch.nsc.ru

D. O. Zharkov (ed.), *Mechanisms of Genome Protection and Repair*, Advances in Experimental Medicine and Biology 1241, https://doi.org/10.1007/978-3-030-41283-8_10

Abbreviations

2D-DIGE	two-dimensional difference gel electrophoresis
3-PGA	3-phosphoglycerate
ACD	1-aminocyclopropane-1-carboxylate deaminase
AOX	alternative oxidase
BADH	Betaine aldehyde dehydrogenase
CMO	Choline monooxygenase
CMP	Comporta
CPS	Carbamoyl-phosphate synthase
DMRL	dimethyl-8-ribityllumazine
ERDL6	the early response to dehydration like 6 protein
ESTs	Expressed sequence tags
FtsH	Filamentation temperature-sensitive H
GB	Glycine betaine
GCS-GS	γ-glutamylcysteine synthetase-glutathione synthetase
GO	Gene Ontology
GS	Glutamine synthetase
GSH	glutathione
GST	glutathione S-transferase
HSPs	Heat shock protein
iTRAQ	isobaric tag for relative and absolute quantification
MG	Methylglyoxal
NMR	the nuclear magnetic resonance
OEI	Oeiras
PAs	polyamines
PC	phytochelatin
PGDH	D-3-phosphoglycerate dehydrogenase
PHP	phosphohydroxypyruvate
PM	plasma membrane
POD	peroxidase
Prx	peroxiredoxins
PTMs	post-translational modifications
PTOX	plastid terminal oxidase
RBOH	NADPH oxidase
RFO	raffinose family of oligosaccharides
ROS	reactive oxygen species
SAMS	S-adenosyl-l-methionine synthetase
SBRM	Sugar beet root maggot
SHMT	serine hydroxymethyl transferase
SOD	superoxide dismutase
SSH	Suppressive subtractive hybridization
VMT	Vaiamonte
Zn	Zinc

10.1 Introduction

In the natural environment, plants are often bombarded by abiotic (such as drought, salt, heat or cold) and biotic (necrotrophic and biotrophic pathogens) stresses (Ku et al. 2018; Kollist et al. 2019). The negative effects of environmental stresses, including abiotic and biotic ones, significantly decrease crop productivity (Anwar et al. 2018). Biotic stress is a significant reason for crop loss and causes yield losses in the range of 31–42%, post-harvest loss due to biotic stress is in the range of 6–20%, and abiotic stress causes 6–20% of the crop damage (Shameer et al. 2018). During the evolution, plants have developed complex strategies that regulate plant developmental, morphological, biochemical and physiological acclimation in order to respond to biotic and abiotic stress (Yongxue et al. 2016; Jian Kang 2016). With the rise of molecular technologies, the mechanisms of plants response to biotic and abiotic stresses including the gene expression patterns, protein patterns and metabolite patterns can be efficiently analyzed by a combination of molecular techniques including genomics, transcriptomics, transgenic technologies, proteomics and metabolomics (Yongxue et al. 2016; Voelckel et al. 2017; Silveira and Carvalho 2016). Recognizing the molecular mechanisms driving plant stress related events and developing molecular strategies to aid plants to tolerate, resist or adapt to biotic and abiotic stress are critical for improving stress tolerance in plants.

Sugar beet (*Beta vulgaris* L.) is a typical vegetative crop with a biennial life cycle, which has become an important source for sugar production in temperate areas of the world (Hossain et al. 2017). Sugar beet is cultivated in different climates in Europe, North America, and increasingly in Asia, South America and recently North Africa, which suggests that sugar beet

cultivars have certain adaptability to different environments and growth conditions (Hossain et al. 2017; Vastarelli et al. 2013). Sugar beet is moderately salt-tolerant and has certain resistance to cold and drought stress. It is not only used in sugar production, but also used in a wide range of industries including the production of bioethanol as a source of renewable energy (Valli et al. 2012), extraction of pectin from sugar beet pulp (Lv et al. 2013) and production of molasses used as antioxidants, sweetener and colorant (Valli et al. 2012). The root vegetable *Beta vulgaris rubra*, otherwise known as red beetroot (referred to as beetroot) has attracted much attention as a health-promoting and disease-preventing functional food (Clifford et al. 2015). In Central and Eastern Europe, red beetroot is a popular vegetable, and commonly used in food industry as a coloring agent (Georgiev et al. 2010). Known for a long time for its beneficial health effects, beetroot and red beetroot extracts possess antihypertensive, hypoglycemic, antioxidant, anti-inflammatory and hepatoprotective activities and is an effective multiorgan tumor suppressing agent in laboratory animals (Sulakhiya et al. 2016; Krajka-Kuźniak et al. 2012; Gezginci-Oktayoglu et al. 2014).

Sugar beet as a cash crop requires careful agronomical practices and breeding for adaptation to biotic and abiotic stresses to maintain its profitability and ability to produce high-quality taproots. The genome sequence of sugar beet was reported by Dohm et al (2014). In this paper, we review the mechanisms of sugar beet response to biotic and abiotic stresses at the levels of physiological change, the genes' functions, transcription and translation. Understanding how sugar beet responds and tolerates biotic and abiotic stresses is important for boosting sugar beet productivity under these challenging conditions. In order to minimize the negative impact of these stresses, studying how sugar beet has evolved stress coping mechanisms will provide new insights and lead to novel strategies for improving the breeding of stress-resistant sugar beet and other crops.

10.2 The Morphological and Physiological Changes of Sugar Beet in Response to Biotic and Abiotic Stresses

Biotic and abiotic stresses affect plant phenotype and physiology. Salt and drought are the main abiotic stresses in sugar beet that have been extensively studied. The phenotypic and physiological changes of sugar beet under salt, drought and various stress conditions in recent years have been summarized (Table 10.S1). The stress tolerance of sugar beet is a complex trait determined by many physiological and metabolic pathways. These results have laid a foundation for understanding the physiological regulatory mechanisms of stress tolerance in *Beta vulgaris*.

10.2.1 The Morphological and Physiological Changes Under Salt Conditions

Fine-tuned and coordinated regulation of transport, metabolism and redox homeostasis allows plants to acclimate to osmotic and ionic stress caused by high salinity. Sugar beet (*Beta vulgaris* ssp. *vulgaris*), cultivar KWS2320 plants were subjected to a final level of 300 mM NaCl for up to 14 days in hydroponics (Hossain et al. 2017). Sugar beet monosomic addition line M14 tolerates salinity of up to 500 mM sodium chloride (NaCl) for 7 days without losing viability (Yang et al. 2012). Sugar beet is a highly salt tolerant crop plant and is therefore an interesting model to study sodium chloride (NaCl) acclimation in crops.

Some of the central components of the cellular antioxidant defense system in sugar beet were identified, and followed their transcriptional response during acclimation to 300 mM NaCl (Hossain et al. 2017).

The salt tolerance of sugar beet was integrated into many aspects, including storing salt ions in old leaves and petioles, increasing the levels of compatible solutes, enhancing the activity of antioxidants and increasing NADP-ME,

Table 10.S1 The physiological changes of sugar beet under stress conditions

Sugar Beet variety	Stress	Physiological index	Regulation	Treatment description	References
Sugar beet (*Beta vulgaris* subsp. vulgaris) cultivar KWS2320	Salt	The H_2O_2 content	The H_2O_2 content in sugar beet leaves decreased during aging but importantly it was always significantly lower in salt-treated tissue compared with controls.	Sugar beet plants were subjected to a final level of 300 mM NaCl for up to 14 days in hydroponics. Tissue was harvested from the fully expanded third to sixth leaves and the whole root after the point of first branching, from five independent experiments at time points 3 h and 339 h (14 day).	[9]
		MDA levels	MDA levels in salt-stressed leaves were below control samples indicating less lipid peroxidation and membrane damage		
		Non-protein thiols (NPTs),	Non-protein thiols (NPTs) were lower in stressed plants than in controls		
		Glutathione (GSH) levels and GSSG levels	GSH levels dropped in stressed plants from 3 h to 14 days after up-salting. Significantly lower GSH levels were observed in stressed plants at 14 days in contrast there was no significant difference in GSSG levels for control and salinized plants.		
		Ascorbate levels (AsA)	Significantly lower ascorbate levels were observed in stressed plants at 27, 123, 171 and 339 h.		
		DHA content	DHA content during stress acclimation was unchanged		
		Activities of SOD, catalase, GR, APX and peroxidase (POD)	Higher SOD activity was detected in stressed leaves, with the highest activity at 14 days with a five-fold increase compared with controls. GR activity was unaffected by saline growth conditions. However all other antioxidant enzyme activities were stimulated under salinity, with total APX activity showing the least increase, followed by total POD activity and catalase. NADPH oxidase was constant in control leaves at 3 h and 14 days, while it decreased by ~ 40% in salt-stressed plants at 3 h after up-salting up to 300 mM NaCl and by > 60% at 14 days.		
Sugar beet (*Beta vulgaris* L.) variety ST13092	Salt	The content of Na^+, K^+ and Cl^-	The highest concentrations of Na^+ and Cl^- were detected in the tissue of petioles and old leaves under 280 mM NaCl. K contents in the tissue of leave, petiole and root decreased rapidly with the increase of NaCl concentrations from 3 mM to 280 mM.	Ten days after planting under hydroponical condition, seedlings were treated with 70, 140, 210 and 280 mM NaCl for 20 days compared with 3 mM NaCl as control. Each treatment contained four seedlings and has six biological repeats.	[21]
		Total N and P	N contents in the tissue of leave, petiole and root decreased rapidly with the increase of NaCl concentrations from 3 mM to 280 mM. P content showed an increasing pattern in these tissues from 3 mM to 280 mM.		
		The contents of indole acetic acid (IAA), abscisic acid (ABA) and gibberellic acid (GA)	IAA and GA contents in leave decreased rapidly with the increase of NaCl concentrations from 70 mM to 280 mM. ABA contents in leave increased rapidly with the increase of NaCl concentrations from 3 mM to 280 mM.		

		Leaves malondialdehyde (MDA) content, relative electrical conductivity	Leaves malondialdehyde (MDA) content and relative electrical conductivity increased rapidly with the increase of NaCl concentrations from 3 mM to 280 mM.		
		The activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione peroxidase (GPX)	The activities of antioxidant enzymes such as SOD, CAT, APX and GPX showed increasing patterns with increase in salt concentrations from 3 mM to 280 mM.		
		Analysis of chlorophyll content, intercellular carbon dioxide concentration, net photosynthetic rate and stomatal conductance	Analysis of chlorophyll content, intercellular carbon dioxide concentration, net photosynthetic rate and stomatal conductance decreased rapidly with the increase of NaCl concentrations from 70 mM to 280 mM.		
		Osmoprotectants such as free amino acids and betaine	Free amino acids and betaine increased in concentration as the external salinity increased from 3 mM to 280 mM.		
		Two organic acids (malate and citrate) involved in tricarboxylic acid (TCA)-cycle	Malate and citrate exhibited increasing contents under salt stress from 3 mM to 280 mM.		
		Activity of Rubisco, phosphoenolpyruvate carboxylase (PEPC), NADP-malate dehydrogenase (NADP-MDH), NADP-malic enzyme (NADP-ME)	Rubisco activity was inhibited under salt stress. The activity of NADP-malic enzyme, NADPmalate dehydrogenase and phosphoenolpyruvate carboxylase showed a trend that first increased and then decreased. Their activities were highest with salinity at 140 mM NaCl.		
Two sugar beet varieties KWS0143 (strong tolerance to saline-alkali) and Beta464 (weak tolerance to saline-alkali)	Saline-alkali	Dry mass,rhizosphere soil enzyme activities and soil microbial quantities	There were significantly positive correlation among the dry mass,soil enzymes and soil microbe of the two varietie. Path coefficient analysis showed the determinant coefficient of KWS0143 dry mass was in order of sctinomycetes > bacteria > peroxidase > urease > fungi > alkali phosphatase, while that Beta464 dry mass was in order of actinomycetes > peroxidase > urease > fungi > alkali.	Pot experiments were designed with three replicates by two sugar beet varieties, KWS0143 and Beta464 planted in different Na_2CO_3 concentrations [0% (control),0.4%, 0.8% and 1.2% of soil].	[22]

(continued)

Table 10.S1 (continued)

Sugar Beet variety	Stress	Physiological index	Regulation	Treatment description	References
Beta vulgaris L.	Salt	Relative fresh weight (RWC), maximum photochemical efficiency (FV/FM)and relative chlorophyll content (SPAD)	While 300 mM NaCl exceeded the optimal levels and caused reduced growth in sugar beet, there were no detrimental effects on RWC, FV/FM or chlorophyll content.	Halophyte (*Vicia faba* L., broad beans; *Beta vulgaris* L., sugar beet; *Pisum sativum* L., pea) and glycophyte species (*Chenopodium* quinoa, Quinoa; Copenhagen Mesembryanthemum purpureus, pig face Bunnings; Disphyma crassifolium, noon-flower)were grown under saline conditions (0 mM, 100 mM and 300 mM NaCl) in a glasshouse	[23]
		Net K+ flux, net H+ flux, amplitude of K+ and H+ responses to light in mesophyll segments with contrasting salinity tolerance exposed to 100 mM NaCl for 24 h	Light/dark transitions resulted in a strong modulation of kinetics of net K^+ and H^+ fluxes across the mesophyll cell plasma membrane. The effect of salinity on the light-induced K^+ flux response from mesophyll was not significant in sugar beet. The magnitude of light/dark modulation of net H^+ flux increased less in sugar compared with bean.		
Maize seeds (*Zea mays* L. cv. Pioneer 3906) and sugar beet plants	Na^+/K^+ (mM) 0/100, 25/75, 50/50, 75/25, 100/0	Na^+/K^+ ratios, plasma membrane (PM) H^+-ATPase activity	At 25 mM Na^+ concentration, PM H^+-ATPase activity was not affected in sugar beet. In maize and sugar beet, reduction in active H^+ flux was 20% and 5% at 25 mM Na^+ concentration in the assay, respectively. The active H^+ flux was decreased to 80% and 60%, when 100 mM K^+ were substituted by 100 mM Na^+.	Maize seeds (Zea mays L. cv. Pioneer 3906) and sugar beet plants were cultured using hydroponic culture in a climate chamber. Plasma membrane (PM) fraction were isolated from maize and sugar beet shoots with two-phase partitioning method.	[24]

Sugar beet (*Beta vulgaris* L.)	Salt	Effect of isolates on shoot length, root length and dry mass of sugar beet plants	After 12 weeks, it was observed that plant shoot length, root length and dry mass of inoculated plants were significantly increased compared with those of the control plants at elevated NaCl concentrations.	Sugar beet seedlings with a similar phenotype were transplanted into pots with 10 kg sterilized rivers and mixed with 20% perlite. One-month-old plants were irrigated with 5 mL bacterial suspension of 108 cells mL^{-1}. Control plants were treated with water without bacterial culture. Following bacterial infection, plants were allowed to grow for 24 h. Plants were then exposed to four different salt concentrations (50, 75, 100 or 125 mM NaCl). NaCl was dissolved in 1 L Hoagland's nutrient solution and 200 mL of this solution was irrigated every day, which equaled the volume that was drained from the pots. Control plants were irrigated with 200 mL nutrient solution. Each treatment contained six plants and had four replicates. At 12 weeks' post-inoculation, the leaf of the plants of each treatment was chosen to analyze.	[34]
		Effect of inoculation with halotolerant bacteria on host ACC(1-aminocyclopropane-1-carboxylate) and ethylene biosynthesis	Salinity induced an increase in ACC concentration and ethylene synthesis in sugar beet tissue. Bacteria-inoculated plants showed clear decreases in both ACC concentration and ethylene synthesis.		
		Effect of isolates on total chlorophyll content and maximum photochemical yield (Fv/Fm) of sugar beet plants.	Although the chlorophyll content and Fv/Fm were reduced with increasing salinity, when bacteria were present there were significant increases in chlorophyll content and Fv/Fm regardless of salt concentration.		

(continued)

Table 10.S1 (continued)

Sugar Beet variety	Stress	Physiological index	Regulation	Treatment description	References
Sugar beet (*Beta vulgaris* cv.Beta 356)	Drought	Malonaldehyde (MDA) content, relative conductivity, catalase (CAT) activity, and soluble sugar content	MDA content, relative conductivity, CAT activity, and soluble sugar content began to increase 24 h after rehydration.	Sugar beet (*Beta vulgaris* cv.Beta 356) was subjected to drought stress during vegetative development by maintaining the soil water content in the 0–40 cm soil depth at 70%, 50% or 30% of field capacity to study the physiological traits of the leaves.	[35]
		Proline content	Proline content began to increase 48 h after rehydration.		
		The activities of peroxidase (POD)	No compensation effect was observed in POD activity after rehydration.		
Sugar beet (*Beta vulgaris* L. cv. Felicita)	Drought	Activities of SOD, APX, POX and CAT	Drought stress stimulated SOD activity in eight out of ten mutants compared with the control. APX activity was enhanced in four out of ten mutants. POX and CAT activities increased significantly in all mutants.	After obtaining shoot tip explants, they were irradiated with 0, 10, 25, 35, 50 and 75 Gy of gamma rays. On day 28, regeneration rates and fresh weights of non-irradiated (as control) and irradiated plants regenerated in tissue cultures were observed and recorded. According to these growth parameters, the GR30 and GR50 doses of shoot tips were determined, from which the optimal radiation dose was chosen to be 20 Gy. After that, the M1V1 generation, which was obtained after explants were irradiated with 20 Gy as optimal radiation dose, was multiplied on the same medium up to M1V3 generations every 28 days. Among the M1V3 plantlets, drought-tolerant mutants were selected on MS medium supplemented with 10^{-2} and 2×10^{-2} kg/L PEG6000.	38
		Ferric ion reducing antioxidant power (FRAP) values, chlorophyll and carotenoid contents	FRAP values, chlorophyll and carotenoid contents were enhanced under stress conditions in all mutant plants compared with the control.		

NADP-MDHN and PEPC activities under moderate salt stress (Wang et al. 2017).

The effect of saline-alkali stress on dry mass, rhizosphere soil enzyme activities and soil microbial quantities were recorded (Guo et al. 2016). The results showed that the numbers of soil microbial communities and the activities of soil enzymes directly affected the stress resistance of sugar beet.

The causal link between mesophyll ion transport activity and plant salt tolerance in a range of evolutionary contrasting halophyte (*Vicia faba* L., Broad beans; *Beta vulgaris* L., Sugar beet; *Pisum sativum* L., Pea) and glycophyte species (*Chenopodium quinoa*, Quinoa; *Mesembryanthemum purpureus*, Pigface; *Disphyma crassifolium*, Noon-flower) were established (Percey et al. 2016). Accordingly, whole-plant physiological and agronomical performance also clearly followed quinoa > sugar beet > bean pattern. In sugar beet, photosynthetic parameters under salinity stress showed no decline. Moreover, in sugar beet the chlorophyll content even increased significantly in plants grown at high NaCl concentrations which was attributed to the ability of mesophyll cells to control cytosolic K^+ homeostasis under the salinity stress conditions.

The effect of different Na^+/K^+ ratios on plasma membrane (PM) H^+-ATPase activity in salt sensitive maize (*Zea mays* L.) and salt-resistant sugar beet shoot were researched in vitro (Wakeel et al. 2011a). An increased Na^+/K^+ ratio decreased the PM H^+-ATPase activity in vesicles from maize as well as from sugar beet shoots. Nevertheless, the detrimental effect of an increased Na^+/K^+ ratio was more severe in salt-sensitive maize compared to salt-resistant sugar beet. Sugar beet expresses specific H^+-ATPases, which were more tolerant of elevated Na^+ concentration as compared to those of salt-sensitive maize. Plant growth is impaired primarily by osmotic stress in the first phase of salt stress (Munns 1993), whereas Na^+ toxicity affects the plant growth mainly in the second phase (Fortmeier and Schubert 1995; Sümer et al. 2004). Salinity leads to an increased Na^+/K^+ ratio and thus displacement of K^+ by Na^+ in the plant cell. Relatively higher cytosolic Na^+ concentrations may have an effect on the activity of plasma membrane (PM) H^+-ATPase. A decreased PM-H^+-ATPase activity could increase the apoplastic pH (Bibikova et al. 1998). This process could limit the cell-wall extensibility and thus reduce growth according to the acid growth theory (Rayle and Cleland 1992; Cosgrove 1997). So, when cytosolic Na^+ accumulation occurs, specific Na^+-tolerant ATPase isoforms may contribute to salt resistance of sugar beet.

Utilization of rhizobacteria associated with plant roots in harsh environments could be a feasible strategy to deal with limits to agricultural production caused by soil salinity (Timmusk et al. 2014). Halophytes occur naturally in high-salt environments, and their roots may be associated with promising microbial candidates for promoting growth and salt tolerance in crops (Jha et al. 2012; Zhao et al. 2016). The efficient halotolerant plant growth-promoting rhizobacterial strains from halophytes were isolated and their activity and effects on sugar beet growth were evaluated under salinity stress (Zhou et al. 2017). Three isolates, identified as *Micrococcus yunnanensis*, *Planococcus rifietoensis* and *Variovorax paradoxus* enhanced salt stress tolerance remarkably in sugar beet, resulting in greater seed germination and plant biomass, higher photosynthetic capacity and lower stress-induced ethylene production at different NaCl concentrations (50–125 mM). These results demonstrated that salinity adapted 1-aminocyclopropane-1-carboxylate deaminase (ACD)-producing bacteria isolated from halophytes could promote sugar beet growth under saline stress conditions.

10.2.2 The Morphological and Physiological Changes Under Drought Conditions

Leaf vascular bundles and palisade tissues of sugar beet are well developed, and the species is a direct root crop with strong drought tolerance (Li et al. 2016). Root yield, sucrose content and juice purity are essential for the efficiency of sugar manufacturing and have been continuously

improved by breeding. To ensure further progress, however, tolerance against biotic and abiotic stress factors such as drought is gaining increasing importance (Jansen and Stibbe 2007). In recent years, drought has been found to be one of the major factors limiting sugar beet yield (Pidgeon et al. 2005).

Sugar beet (*Beta vulgaris* cv. Beta 356) was subjected to drought stress during the vegetative development by maintaining the soil water content in the 0–40 cm soil depth at 70%, 50% or 30% of field capacity to study the physiological traits of the leaves (Li et al. 2016). The results showed that the compensation index was at the highest in the 50% field capacity treatment. Supplemental irrigation should be carried out promptly when the soil water content drops to 50% of field capacity during vegetative development. Rehydration could promote self-repair functions in leaves, thus reducing the effects of drought on sugar beet yield and sugar content.

Mutation induction with gamma radiation via in vitro tissue culture is a useful technique for improving plant tolerance against environmental stresses. Putative drought-tolerant sugar beet plants were obtained in in vitro tissue cultures via mutation induction using 20 Gy gamma irradiation. The changes in the antioxidant enzyme systems that were detected both spectrophotometrically and on the level of isoenzyme variations in the earlier stages of development have the ability to determine the differences between the mutant plants and the control. It is hoped that the data presented here will be valuable for future studies on genotype selection, plant development and characterization of gene sources in sugar beet (Sen and Alikamanoglu 2014).

10.2.3 The Morphological and Physiological Changes Under Other Stress Conditions

The oxidative damage caused by light stress in plants and the consequent involvement of pigments are widely studied. The nuclear magnetic resonance (NMR) spectroscopy can be utilized to compare crude leaf extract at different levels of light stress, allowing the analysis of these compounds (Tiziani et al. 2006). The analysis of the ^{1}H NMR (1D) spectra of two agronomic species (*Spinacia oleracea* and *Beta vulgaris*) exposed to different light intensities was presented (Guadagno et al. 2013). In particular, changes in carotenoids and xanthophylls signals were analyzed. NMR spectroscopy provides a powerful complementary way for the identification and quantitative analysis of sugar beet and other plant metabolites either in vivo or in tissue extracts induced by light stress.

Phenol-dependent (guaiacol) peroxidase (POD) is a well-known antioxidant enzyme that protects plant cells from oxidative damage. The activity of POD and its isozyme composition at different stages of the red beet root development were investigated and simultaneously the activity and isozyme composition of POD under hyperosmotic stress and infection of roots by microorganisms were analyzed (Pradedova et al. 2014). The data showed that the activity and isozyme composition of the vacuolar POD, similarly to PODs of other cell compartments changed at different stages of sugar beet development and under stress conditions. So, the changes in the activity and isozyme composition of the vacuolar POD suggest that the central vacuole is not only actively involved in the metabolic processes associated with plant growth and development but is also an important component of cell defense in different types of stress.

The establishment of stress resilient sugar beet is an important breeding goal since this cash crop is susceptible to drought and salinity. The genetic diversity in cultivated sugar beets is low and the beet wild relatives are useful genetic resources for tolerance traits (Fénart et al. 2008; Panella and Lewellen 2007). Three wild beet populations (*Beta vulgaris* ssp. *maritima*) from contrasting environments, Vaiamonte (VMT, dry inland hill), Comporta (CMP, marsh) and Oeiras (OEI, coastland), and one commercial sugar beet (Isella variety, SB), were compared. At the genetic level, the use of six microsatellite allowed to detect a total of 76 alleles. The physiological responses of the populations under drought and salt stress, namely at initial stress stages, late stress stages, and early stress recovery were evaluated. Two striking ecotypes were VMT, which was the best to cope with

drought and salinity, and CMP which had the highest root to shoot ratio (Ribeiro et al. 2016). These genotypes can supply breeding programs with distinct goals.

10.2.4 The Tolerance and Adaptation Mechanisms in Physiological Changes of Sugar Beet in Response to Biotic and Abiotic Stresses

Reactive oxygen species (ROS) accumulation is a common denominator under conditions of stress (Foyer et al. 1994). Complementary to the generator systems are enzymatic and non-enzymatic antioxidants that regulate ROS and redox homeostasis and counteract metabolic imbalances, damage to cell structures, cell death and stress adaptation (Foyer et al. 2009). It was concluded that the increase in the content of enzymes and non-enzymatic antioxidants were also the main strategies for sugar beet to regulate ROS and redox homeostasis under salt, drought and various stresses.

The ability of maintaining a stable Na^+/K^+ ratio and cytosolic K^+ homeostasis were the main factors in the sugar beet response to salt stress. The increase in proline is one of the main physiological indicators of sugar beet response to drought stress. The stress tolerance potential of sugar beet was improved by crossbreeding with wild type sugar beet, mutation breeding and inoculating rhizobacteria from the rhizosphere of halophytes to the root of sugar beet.

10.3 The Functions of Genes from Sugar Beet in Response to Stresses

Transgenic technology is an important method to study gene functions. Recently, studies on the function of stress-responsive genes in sugar beet were mainly focused on salt stress. The functions of genes responding to cold and heavy metal stress have also investigated. These genes in sugar beet will provide potential candidate genes for improving stress resistance in sugar beet and other crops.

10.3.1 The Functions of Genes from Sugar Beet in Response to Salt Stress

Sugar beet is an economically important, salt-tolerant, and widely distributed plant, which can grow under 300 mM NaCl and even 500 mM treatment (Hossain et al. 2017; Yang et al. 2012). It accumulates glycine betaine (GB) as an osmoprotectant under NaCl stress and is an excellent model plant to study the mechanisms of salt stress. Several salt-stress-related genes have been isolated from sugar beet (Russell et al. 1998; Tabuchi et al. 2006).

Serine biosynthesis in plants proceeds through two pathways: the phosphorylated pathway and the photorespiratory pathway (Ros et al. 2014). In the photorespiratory pathway, serine is synthesized from glycine in the presence of serine hydroxymethyl transferase (SHMT) (Ros et al. 2014). In the phosphorylated pathway, serine is derived from 3-phosphoglycerate (3-PGA). D-3-phosphoglycerate dehydrogenase (PGDH) catalyzes the oxidation of 3-PGA to form phosphohydroxypyruvate (PHP) by utilizing NAD as a cofactor (Ros et al. 2014). One study investigated the significance of serine biosynthetic genes for salt stress in sugar beet (Kito et al. 2017). mRNA transcriptional expression for *BvPGDHa* was significantly enhanced under salt stress conditions in both leaves and roots of sugar beet. On the other hand, *BvSHMTa* was expressed transiently in leaves and roots under salt stress. PGDH activity was high in storage root. After salt stress, PGDH activity was increased in leaf, petiole, and root. Recombinant proteins were expressed in *Escherichia coli*. The results suggest that *BvPGDHa* and *BvSHMTa* play an important role in salt tolerance.

Sugar beet accumulates large amount of GB in the leaves as well as in the storage roots (Hanson and Wise 1982). GB is an important osmoprotectant and is synthesized by two-step oxidation of choline (Takabe et al. 2006). Choline monooxygenase (CMO) catalyzes the first step of the pathway and is believed to be a rate-limiting step for GB synthesis (Rathinasabapathi et al. 1997). Since many crop plants do not have the GB biosynthetic pathway, genetic engineering of this

pathway could be useful to improve stress tolerance (Rontein et al. 2002). In order to investigate the role of CMO for GB accumulation in sugar beet, transgenic plants carrying the antisense *BvCMO* gene were developed (Yamada et al. 2015). The antisense *BvCMO* plants showed the decreased activity of GB synthesis from choline compared to wild-type (WT) plants, which was well related to the suppressed level of *BvCMO* protein. However, GB contents were similar between transgenic and WT plants with the exception of young leaves and storage roots. Transgenic plants showed enhanced susceptibility to salt stress compared with WT plants. These results suggest the importance of choline precursor supply for GB accumulation, and young leaves and storage root as sensitive sites for GB accumulation.

Na^+/H^+ exchange (NHX) activity was correlated with the salinity tolerance of *B. vulgaris* cell cultures (Blumwald and Poole 1987). A cDNA encoding BvNHX1 cation exchanger protein was isolated (Xia et al. 2002). The *BvNHX1* transcript levels in both the suspension cell culture and whole plants increased following salt treatment and this increase was concomitant with the elevated BvNHX1 protein and vacuolar Na^+/H^+ antiporter activity (Xia et al. 2002). The promoter of *BvNHX1* was cloned and its activity was studied in transgenic *Arabidopsis* expressing the *BvNHX1::GUS* construct (Adler et al. 2010). The minimal 337 bp promoter sequence that was upregulated by salt treatment and osmotic stress was identified by constructs containing serial deletions of the promoter. Mutating four putative *cis*-acting elements within the 337 bp promoter fragment revealed that MYB transcription factor(s) is involved in the activation of the expression of *BvNHX1* upon exposure to salt and water stresses.

Sugar beet M14 line is an interspecific hybrid crossed between cultivated sugar beet *B. vulgaris* L. and wild species *B. corolliflora* Zoss., which contains the 9th chromosome of *B. corolliflor*a and the complete *B. vulgaris* genome (Guo et al. 1994). It has several interesting characteristics including apomixis and tolerance to drought, cold and salt stress (Yang et al. 2012). Sugar beet M14 therefore can function as a unique germplasm for isolating genes from the wild species for improvement in cultivated beet. The functions of three genes (*BvM14-glyoxalase* I, *BvM14-cystatin* and *BvM14-SAMS2*) in response to salt stress were studied in sugar beet M14.

Methylglyoxal (MG) is a by-product of several metabolic pathways that are involved in the degradation of carbohydrates, proteins and lipids (Hoque et al. 2016). However, under environmental stresses, both biotic and abiotic, MG production is abruptly increased making its levels highly toxic to cellular biomolecules, including proteins, DNA, RNA and lipids (Bilova et al. 2017). To combat MG toxicity, plants contain a powerful glyoxalase system comprising glyoxalase I and II, the enzymes that detoxify excessive MG using glutathione (GSH) as co-factor (Kaur et al. 2017; Ghosh et al. 2014). The *BvM14-glyoxalase* I gene from sugar beet M14 is ubiquitously expressed in different tissues of sugar beet M14 line and upregulated in response to salt, mannitol and oxidative stresses. Heterologous expression of *BvM14-glyoxalase* I increases *E. coli* tolerance to methylglyoxal. Transgenic tobacco plants constitutively expressing *BvM14-glyoxalase* I were generated. Both leaf discs and seedlings showed significant tolerance to methylglyoxal, salt, mannitol and H_2O_2. These results suggest an important role of *BvM14-glyoxalase* I in cellular detoxification and tolerance to abiotic stresses.

Constitutive expression of *Arabidopsis* cystatins in yeast and *Arabidopsis* seedlings increased their tolerance to high salt, drought, oxidative, and cold stresses (Zhang 2008). Overall, cystatins may play a crucial role in plant stress tolerance and defense (Van der et al. 2003). *BvM14-cystatin* from sugar beet M14 was expressed ubiquitously in roots, stems, leaves and flower tissues with relatively high abundance in developing stems and roots (Wang et al. 2012). It was found to be localized in the nucleus, cytoplasm and plasma membrane. Recombinant BvM14-cystatin expressed in *E. coli* was purified and exhibited cysteine protease inhibitor activity. Salt-stress treatment induced *BvM14-cystatin* transcript levels in the M14 seedlings. Homozygous *Arabidopsis* plants over-expressing *BvM14-cystatin* showed enhanced salt toler-

ance. Taken together, these data improved understanding of the functions of *BvM14-cystatin* and highlighted the possibility of employing the cystatin in engineering plants for enhanced salt tolerance.

S-adenosyl-L-methionine synthetase (SAMS), one of the salt-responsive genes, is an important enzyme in the synthesis of SAM. Usually, SAM synthesized by SAMS from methionine and ATP acts as a universal methyl group donor involved in numerous transmethylation reactions (Roje 2006). It plays a vital role in metabolism and development regulation, as well as in abiotic and biotic stress responses (Nagel et al. 2008; Köllner et al. 2010). Additionally, it functions as a precursor for the synthesis of polyamines (PAs), which are involved in regulating plant responses to abiotic or biotic stresses (Jang et al. 2012). To determine the potential role of SAMS from sugar beet in salt tolerance, *BvM14-SAMS2* gene from the salt-tolerant sugar beet M14 was isolated (Ma et al. 2017). The expression of *BvM14-SAMS2* in leaves and roots was greatly induced by salt stress. Overexpression of *BvM14-SAMS2* in *Arabidopsis* resulted in enhanced salt and H_2O_2 tolerance. Furthermore, a knock-down T-DNA insertion mutant of *AtSAMS3*, which shared the highest homology with *BvM14-SAMS2*, was obtained Interestingly, the mutant *atsam3* showed sensitivity to salt and H_2O_2 stress. The result showed that the antioxidant system and polyamine metabolism played an important role in salt and H_2O_2 tolerance in the *BvM14-SAMS2* overexpressed plants. The results had provided new insights into SAMS functions in sugar beet.

Plants acclimate to NaCl stress by maintaining its growth rate and adjusting its cellular redox and ROS network. In order to understand the mechanisms of ROS accumulation and scavenging in sugar beet under severe salinity, the regulation of key enzymatic antioxidants involved in the redox and ROS network was investigated at the transcript level. Sugar beet plants were subjected to a final level of 300 mM NaCl for up to 14 days in hydroponics (Hossain et al. 2017). First, the gene families of superoxide dismutase (SOD), peroxiredoxins (Prx), alternative oxidase (AOX), plastid terminal oxidase (PTOX) and NADPH oxidase (RBOH) were identified in the sugar beet genome (Hossain et al. 2017). Salinity induced the accumulation of *Cu-Zn-SOD*, *Mn-SOD*, *Fe-SOD3*, all *AOX* isoforms, 2-*Cys-PrxB*, *PrxQ*, and *PrxIIF* at the transcriptional level (Hossain et al. 2017). In contrast, *Fe-SOD1*, 1-*Cys-Prx*, *PrxIIB* and *PrxIIE* levels decreased in response to salinity at the transcriptional level. Most importantly, *RBOH* transcripts of all isoforms decreased (Hossain et al. 2017). The effect of salt stress on *BvpAPX* expression in leaves of the cultivated beet varieties, Huzar and Janosik, and their wild salt-tolerant relative *B. vulgaris* ssp. *maritima* were determined (Dunajska et al. 2014). Plants were subjected to salt stress during a 32-day culture period (long-term salt treatment). An alternative salinization protocol consisted of an 18-h incubation of detached beet leaves in media supplemented with toxic salt concentrations (short-term salt treatment). qRT-PCR analysis revealed that *BvpAPX* expression markedly increased in leaves of plants subjected to conditions of long-term treatment with salinity, whereas *BvpAPX* transcript levels remained unaffected in detached leaves during short-term salt treatment. It is possible that assessment of the timing of transcriptional activation of the *BvpAPX* gene in plants challenged with long-term treatments with salinity may provide cues for screening beet genotypes for salt tolerance.

10.3.2 The Functions of Genes from Sugar Beet in Response to Cold Stress and Heavy Metal Stress

An early response to dehydration like 6 protein (ERDL6) in *Arabidopsis* was demonstrated directly to operate as a proton-driven glucose exporter involved in glucose export from vacuoles into the cytosol (Poschet et al. 2011). Overexpression of *BvIMP*, the closest sugar beet homolog to *AtERDL6*, in *Arabidopsis* affected glucose concentrations under cold conditions and

led, surprisingly, to impaired seed germination under both conditions, sugar application and low environmental temperatures (Klemens et al. 2014). Upon cold treatment, *BvIMP* overexpressing plants accumulated lower quantities of monosaccharides than the wild-type plants, a response in line with the reduced frost tolerance of the transgenic *Arabidopsis* plants, and the fact that cold temperatures inhibited *BvIMP* transcription in sugar beet leaves. These findings showed that the tight control of vacuolar sugar import and export was a key requisite for cold tolerance and seed germination of plants.

Sugar beet is an emerging renewable energy crop with high biomass and ethanol conversion rate and a strong ability to adapt to the environment, making it a suitable target species for phytoremediation which is a highly efficient, economical and environmentally friendly way to remove heavy metals from contaminated soil (Reeves and Baker 2000). During the remediation process, these toxic elements are extracted or stabilized by plants and metabolized in their tissues (Reeves and Baker 2000). A novel *Streptococcus thermophiles* γ-glutamylcysteine synthetase-glutathione synthetase (StGCS-GS) that synthesizes GSH with limited feedback inhibition was overexpressed in sugar beet, yielding three transgenic lines with enhanced tolerance to different concentrations of cadmium, zinc and copper, as indicated by their increased biomass, root length and relative growth compared with wild-type plants (Liu et al. 2015). Transgenic sugar beets accumulated more Cd, Zn and Cu ions in shoots than wild-type, as well as higher GSH and phytochelatin (PC) levels under different heavy metal stresses. This enhanced heavy metal tolerance and increased accumulation were likely due to the increased expression of StGCS-GS and consequent overproduction of both GSH and PC. The results demonstrate the explicit role of StGCS-GS in enhancing Cd, Zn and Cu tolerance and accumulation in transgenic sugar beet, which may represent a highly promising new tool for phytoremediation.

10.3.3 The Molecular Mechanisms of Genes from Sugar Beet in Response to Stresses

The functions of 15 genes related to sugar beet in response to salt, cold and heavy metal stresses were summarized by transgenic technologies or the transcriptional expression level, providing a good basis for further exploitation and utilization of stress-resistant gene resources in sugar beet.

10.4 The Transcriptome Analysis of Sugar Beet in Response to Biotic and Abiotic Stresses

Plant transcriptomics is widely used in studying plant responses to various stresses. Transcriptomic studies have revealed many changes in expression levels of various genes during exposure to environmental extremes (Imadi et al. 2015). Transcriptomics are applied widely in plants to help us understand physiological and molecular responses in terms of genome sequence, gene regulation, gene differentiation, posttranscriptional modifications and gene splicing and are used to quantify the modulations in gene expression levels during different stress conditions and developmental stages (Wang et al. 2009). Sugar beet transcriptome have recently received some impulse, which have focused on exposure to multi-stress conditions including salt, cold and other stresses.

10.4.1 The Transcriptome Analysis of Sugar Beet in Response to Salt Stress

Previous studies reported that sugar beet monosomic addition line M14 obtained from the intercross between *Beta vulgaris* L. (cultivated species) and *B. corolliflora* Zoss (wild species) exhibited tolerance to salt (up to 0.5 M NaCl) stress (Yang et al. 2012). Comparative transcriptomics was performed to monitor genes differen-

tially expressed in the leaf and root samples of the sugar beet M14 seedlings treated with 0, 200 and 400 mM NaCl, respectively (Lv et al. 2018). Digital gene expression revealed that 3856 unigenes in leaves and 7157 unigenes in roots were differentially expressed under salt stress. Enrichment analysis of the differentially expressed genes based on GO and KEGG databases showed that in both leaves and roots genes related to regulation of redox balance, signal transduction, and protein phosphorylation were differentially expressed. Comparison of gene expression in the leaf and root samples treated with 200 and 400 mM NaCl revealed different mechanisms for coping with salt stress. In addition, the expression levels of nine unigenes in the ROS scavenging system exhibited significant differences in the leaves and roots. The transcriptomics results have provided new insights into the salt-stress responses in the leaves and roots of sugar beet.

Sea beet (*Beta vulgaris* ssp. *maritima*) is a wild relative of cultivated beets (*Beta vulgaris*) which displays elevated salt stress-resistance, compared to other beet varieties. It is reported that sea beet has higher osmotic adjustment value and higher leaf succulence index compared to the cultivated beets (Bagatta et al. 2008). The transcriptome response to acute salt stress imposed to excised leaves of sea beet was investigated (Skorupa et al. 2016). Sequencing libraries were generated from leaves subjected to either moderate (150 mM NaCl) or strong (300 mM NaCl) salt stress. Control libraries were constructed from untreated leaves. Sequencing was performed using the Illumina MiSeq platform. 32,970 unigenes were obtained by assembling the pooled reads from all the libraries with Trinity software. Screening the nr database returned 18,362 sequences with functional annotation. Using the reference transcriptome, 1246 genes that were differentially expressed after 48 hours of NaCl stress were identified. Genes related to several cellular functions such as membrane transport, osmoprotection, molecular chaperoning, redox metabolism or protein synthesis were differentially expressed in response to salt stress. The response of sea beet leaves to salt treatments was marked out by transcriptomic upregulation of genes related to photosynthetic carbon fixation, ribosome biogenesis, cell wall building and cell wall expansion.

10.4.2 The Transcriptome Analysis of Sugar Beet in Response to Cold Stress

The reference transcriptome assembly has been published for sugar beet adult plants under gibberellin treatment and vernalization (Mutasa-Go¨ttgens et al. 2012) and for elucidating the pathway of accumulation of sucrose in taproots (Turesson et al. 2014).

Low-temperature stress is a significant cause of sugar beet quality and production losses in European and North American agriculture (Kirchhoff et al. 2012). The survival of young sugar beet plantlets and the subsequent sucrose yield of mature plants are often seriously limited by low temperatures, especially when plantlets are exposed to freezing temperatures (below 0 °C) at early developmental stages (Kirchhoff et al. 2012). The wide study of sugar beet transcriptome modulation after a short exposure to a cold stress, mimicking what was experienced in vivo by young plantlets when temperature droped in the early spring nights, was carried out by high-throughput sequencing of leaves and root RNAs (RNA-Seq) (Moliterni et al. 2015). In total, approximately 200 million of Illumina paired-end reads were obtained and 20,927 unique contigs with a read count of at least 100 reads per contig were assembled, with an average length of 2161 bp. When comparing the transcriptome of control and low temperature-treated sugar beet plants, a total of 549 (451 positively and 98 negatively regulated) differentially expressed sequences in leaves and 656 (555 positively and 101 negatively) in roots were retrieved. A significant picture of the earliest events of temperature sensing was achieved for the first time for sugar beet: the retrieval of a great amount of transcription factors and the intensity of modulation of a large number of genes involved in several metabolic pathways suggested a fast and deep rearrangement of sugar beet plantlets

metabolism as early response to cold stress, with both similarities and specificities between the leaf and root tissues.

10.4.3 The Transcriptome Analysis of Sugar Beet in Response to Biotic Stress

Suppressive subtractive hybridization (SSH) is a technique used to identify differentially expressed genes in cells important for growth and differentiation at the transcriptome level, which has often been used to study molecular mechanisms of plants in biotic and abiotic stresses (Lukyanov et al. 2007; Sahebi et al. 2015). The response to insect pests in the root of sugar beet is an interesting area of plant defense research. More than 150 sugar beet root ESTs (Expressed sequence tags) were identified enriched for genes that responded to feeding by the sugar beet root maggot (SBRM) in both the moderately resistant genotype F1016 and the susceptible F1010 using SSH (Puthoff and Smigocki 2007). Of the examined ESTs, 20% were regulated by methyl jasmonate, 17% by salicylic acid and 11% by ethylene, suggesting that these signaling pathways were involved in sugar beet root defense response. Identification of these sugar beet root ESTs provided knowledge concerning plant root defense and would likely lead to the development of novel strategies for the control of the sugar beet root maggot. This research provided significant knowledge about how sugar beet protect itself against insect invasions.

Interestingly, there is a complementary transcriptome analysis of sugar beet root maggot (SBRM) (*Tetanops myopaeformis*) genes modulated by the infestation of sugar beet roots (Li and Smigocki 2018). SRBM, the most destructive insect pest of *Beta vulgaris* host, is the larva of a small fly, affecting more than half of all North American sugar beet acreage. SSH generated more than 300 SBRM ESTs differentially expressed in the interaction of the pest with a moderately resistant (F1016) and a susceptible (F1010) sugar beet line. Gene Ontology (GO) analysis predicted a dominance of metabolic and catalytic genes involved in the interaction of SBRM with its host. The functions of SBRM genes during development, regulation, cellular process, signaling and under stress conditions were annotated. This research provided the information on how insects evolve adaptive mechanisms to overcome host sugar beet resistance and develop tolerance to many insecticides used for their control.

10.4.4 The Tolerance and Adaptation Mechanisms in Transcriptomic Changes of Sugar Beet in Response to Biotic and Abiotic Stresses

More than 10,000 unigenes were differentially expressed under salt stress, 1205 unigenes were differentially expressed under cold stress, 150 ESTs from sugar beet and 300 ESTs from SBRM were profiled, which revealed the molecular mechanism of sugar beet in response to biotic and abiotic stresses in transcriptomic changes.

10.5 The Proteome Analysis of Sugar Beet in Response to Biotic and Abiotic Stresses

Because of post-transcriptional events and post-translational modifications such as phosphorylation and glycosylation, it is of essential importance to study stress responses at the protein level. As a necessary and complementary approach in the postgenomic era, proteome analysis is a relatively new and a powerful set of tools for studying large-scale plant responses to biotic and abiotic stresses (Pandey and Mann 2000; Wakeel et al. 2011b). Recent analyses of proteomes in sugar beet in response to salt stress and other stresses have yielded more information for understanding the complex mechanisms of sugar beet stress response and tolerance (Table 10.1).

Table 10.1 The proteomic analysis of sugar beet under stress conditions (Summary of publications on sugar beet stress responsive proteomics)

Sugar Beet variety	Stress	Tissue	Treatment description	Proteomics technique	Differentially expressed proteins (unique proteins)	References
Sugar beet (*Beta vulgaris* L. cv. Evita)	Salt	Shoot	Sugar beet plants were grown in hydroponics under 1 mM NaCl (control) and 125 mM NaCl treatments. Plants were harvested 7 d after salt treatment. The experiment was replicated three times with 70 plants per treatment.	Two-dimensional gel electrophoresis (2-DE)	6 (4 up-regulated,2 down-regulated)	[91]
		Root			3 (3 up-regulated)	
Sugar beet monosomic addition line M14	Salt	Leave	The M14 seeds were sterilized and then sown in vermiculite for germination and watered daily. After 1 week, seedlings were transferred to hydroponic containers containing Hoagland solution. Seedlings were grown in a growth chamber. Salt treatment was initiated 3 weeks after sowing. Two NaCl concentrations were applied: 0 (control) and 500 mM. To avoid osmotic shock, salt was gradually increased by 50 mM each day until the desired concentration was reached. Plants were harvested 7 days after salt treatment. Three independent biological replicates, each with leaves or roots from plants were prepared.	Two-dimensional difference gel electrophoresis (2D-DIGE)	38	[20]
		Root			29	
Sugar beet monosomic addition line M14	Salt	Leave	The M14 seeds were sterilized and then sown in vermiculite for germination and watered daily. After 1 week, seedlings were transferred to hydroponic containers containing Hoagland solution. Seedlings were grown in a growth chamber. Salt treatment was initiated 3 weeks after sowing. Three NaCl concentrations were applied: 0 (control), 200 mM and 400 mM. To avoid osmotic shock, salt was gradually increased by 50 mM each day until the desired concentration was reached. Plants were harvested 7 days after salt treatment. Three independent biological replicates, each with leaves or roots from plants were prepared.	2D-DIGE and isobaric tag for relative and absolute quantification (iTRAQ)	142	[93]
		Root			65	
Sugar beet monosomic addition line M14	Salt	Leave	The M14 seeds were sterilized and then sown in vermiculite for germination and watered daily. After 1 week, seedlings were transferred to hydroponic containers containing Hoagland solution. Seedlings were grown in a growth chamber. Salt treatment was initiated 3 weeks after sowing. Three NaCl concentrations were applied: 0 (control), 200 mM and 400 mM. To avoid osmotic shock, salt was gradually increased by 50 mM each day until the desired concentration was reached. Plants were harvested 7 days after salt treatment. Three independent biological replicates, each with leaves from plants were prepared.	iTRAQ	50 (40 up-regulated,10 down-regulated)	[96]

(continued)

Table 10.1 (continued)

Sugar Beet variety	Stress	Tissue	Treatment description	Proteomics technique	Differentially expressed proteins (unique proteins)	References
Sugar beet monosomic addition line M14	Salt	Leave	The M14 seeds were sterilized and then sown in vermiculite for germination and watered daily. After 1 week, the seedlings were transferred to hydroponic containers containing the Hoagland solution. Seedlings were grown in a growth chamber. Salt treatment was initiated 3 weeks after sowing. Three NaCl concentrations were applied: 0 (control), 200 mM and 400 mM for 0, 10, 30, 60, 90 min. Salt was added the desired concentration one time. Leaves of M14 seedlings were harvested directly after salt treatment. Three independent biological replicates, each with leaves from plants were prepared.	Label free	114 proteins; 189 phosphoproteins at the phosphorylation level	[100]
Beta vulgaris (sugar beet) and *Beta maritima* (wild beet)	Salt	Leave	Seeds of sugar beet *B.vulgaris cv. felicita* and wild beet *B. maritima* were sown into the pots filled with perlite and grown in a growth chamber. After germination seedlings were watered with ½ Hoagland solution in 3 days interval. Stress treatment started when seedlings were 40 days old by the addition of 250 and 500 mM NaCl into ½Hoagland solution. Control groups were watered only with ½ Hoagland solution. Leaf samples were taken on days 0, 7, 14 and 21 after salt treatment and then used for ChIP procedure immediately. Four independent biological replicates, each with leaves from plants were prepared.	Chromatin Immunoprecipitation (ChIP)	The acetylation patterns were remarkably different between two species in which the highest acetylation levels were found at H3K9 and H3K27 in wild beet and sugar beet respectively.	[103]
Sugar beet genotype (210)	K^+/Na^+	Leave	The seeds of sugar beet genotype (210) were sterilized and germinated in vermiculite with daily watering. After 6 days, the seedlings were transferred into a hydroponic container of pH 5.8 half-strength modified Hoagland solution which did not contain KNO_3, had $NH_4H_2PO_4$ substituted for KH_2PO_4. The plants were grown in a growth chamber. Plants were grown at three K/Na treatments (K was added as KCl, and Na as NaCl), where the K/Na concentration in the initial solution was: 0.03 and 0 mmol/L respectively (K deficiency group); 0.03 and 2.97 (K-Na replacement group), and 3.00 and 0 (control group). Each treatment was labeled T^{def}, T^{rep}, and control. Three independent biological replicates, each with leaves from 8 seedlings were prepared.	2-DE	27	[104]

Sugar beet (*Beta vulgaris* L. cv. Orbis)	Fe deficiency and Fe resupply	Leave	Seeds were germinated and grown in vermiculite for 2 weeks. Seedlings were grown for an additional 2 weeks period in half-strength Hoagland nutrient solution with 45 μM Fe(III)-EDTA, and then transplanted to 20 L plastic buckets (four plants per bucket) containing half-strength Hoagland nutrient solution with either 0 or 45 μM Fe(III)-EDTA. The plants were grown in a growth chamber. Four independent biological replicates, each with young leaves from plants grown for 10d in the presence and absence of Fe were prepared. In the Fe-resupply experiment, 45 μM Fe(III)-EDTA was added to the nutrient solution of plants grown for 10d in the absence of Fe. The apoplastic fluid of these plants was collected 24 h after Fe addition.	2-DE	109	[107]
Sugar beet (*Beta vulgaris* L. "Monohil" and "Orbis"	Fe deficiency and Fe resupply	Root tips	After seed germination in vermiculite and 2 weeks in half-strength Hoagland's nutrient solution with 45 μM Fe(III)-EDTA, plants were transferred into 20 L plastic buckets (four plants per bucket) containing half strength Hoagland's nutrient solution with either 0 or 45 μM Fe(III)-EDTA. In the Fe resupply experiments, plants grown for 10 days in the absence of Fe were transferred to 20 L plastic buckets containing half strength Hoagland's nutrient solution, pH 5.5, with 45 μM Fe(III)-EDTA. The root subapical region from Fe-sufficient plants (+Fe), Fe-deficient plants (-Fe), Fe-deficient plants resupplied with Fe for 24 h (24 h) and Fe-deficient plants resupplied with Fe for 72 h (72 h) was collected. Samples were taken at approximately 4 h after light onset in the growth chamber.	2-DE	22	[112]
Sugar beet (*Beta vulgaris* L. cv. Orbis)	Zn	Root	The plants were grown in a growth chamber. Seeds were germinated and grown in vermiculite for 2 weeks. Seedlings were grown for an additional 2 week period in half-strength Hoagland nutrient solution with 45 μM Fe(III)-EDTA, and then transplanted to 20 L plastic buckets (four plants per bucket) containing half-strength Hoagland nutrient solution with 45 μM Fe(III)-EDTA and different concentrations of Zn. A concentration of 1.2 μM ZnSO4 was used as a control, and excess Zn treatments were 50, 100 and 300 μM ZnSO4. Roots were harvested 9–10 days after imposing the Zn treatments.	2-DE	41	[116]

10.5.1 The Proteome Analysis of Sugar Beet in Response to Salt Stress

The changes in protein expression caused by salinity were monitored using two-dimensional gel-electrophoresis (2-DE) (Wakeel et al. 2011b). Most of the detected proteins in sugar beet showed stability under salt stress. The statistical analysis of detected proteins showed that the expression of only six proteins from shoots and three proteins from roots was significantly altered. At this stage, the significantly changed proteins expression detected could not be attributed to sugar beet adaptation under salt stress. However, unchanged membrane bound proteins under salt stress did reveal the constitutive adaptation of sugar beet to salt stress at the plasma membrane level.

The salt-tolerant mechanism of a special salt-tolerant germplasm resource, sugar beet M14, was studied in depth through a series of proteomic experiments. Sugar beet monosomic addition line M14 displayed interesting phenotypes such as apomixis and salt stress tolerance (Yang et al. 2012; Li et al. 2009). The M14 seedlings were grown in hydroponics with different NaCl concentrations (0 mM, 100 mM, 200 mM, 300 mM, 400 mM, 500 mM, 600 mM, 700 mM, 800 mM, 900 mM) (Yang et al. 2012). Morphological changes were observed 7 days after salt treatment (Yang et al. 2012). The M14 seedlings could survive 7 days of 500 mM NaCl treatment. Some M14 seedlings died at concentrations over 500 mM NaCl, and all of the M14 died at 900 mM NaCl. Therefore, 500 mM NaCl was a high limit concentration that M14 can survive, and it was used in this study to investigate high salt responsive proteins. The proteomic analysis of M14 leaves and roots under 500 mM NaCl treatment for 7 days were reported (Yang et al. 2012). Proteins from control and treated samples were extracted and separated using 2-DE. A total of 40 protein spots from leaf gels and 36 protein spots from root gels exhibited significant changes. Using mass spectrometry and database searching, 38 unique proteins in leaves and 29 unique proteins in roots were identified. The proteins included those involved in metabolism, protein folding, photosynthesis, and protein degradation. This research has revealed candidate proteins for detailed functional characterization, and set the stage for further investigation of the high concentration salt tolerance mechanisms in sugar beet M14. The differentially expressed proteins such as SAMS, glutamine synthetase (GS), carbamoyl-phosphate synthase (CPS), betaine aldehyde dehydrogenase (BADH), ferritin, heat shock proteins (HSPs), filamentation temperature-sensitive H (FtsH), proteasome proteins, glutathione S-transferase (GST) and 14-3-3s have provided interesting candidates for detailed characterization of their functions in salt stress tolerance. This study provided the mechanism of salt tolerance in sugar beet M14 under long-term (7 days) and high salt stress (500 mM NaCl) at the protein level.

In order to understand the tolerance mechanisms of sugar beet M14 under moderate salt stress (200 mM, 400 mM NaCl), the salt-responsive characteristics of the M14 plants under 0, 200, and 400 mM NaCl conditions using quantitative proteomics approaches were reported (Yang et al. 2013). Combining two-dimensional difference gel electrophoresis (2D-DIGE) and isobaric tag for relative and absolute quantification (iTRAQ) technologies, 142 differentially expressed proteins in leaves and 65 differentially expressed proteins in roots were identified under salt stress (Yang et al. 2013). The proteins were mainly involved in photosynthesis, energy metabolism, protein folding and degradation, and stress and defense. This study provided the mechanism of salt tolerance in sugar beet M14 under long-term (7 days) and moderate salt stress (200 mM, 400 mM NaCl) at the protein level, including enhancement of photosynthesis and energy metabolism, accumulation of osmolyte and antioxidant enzymes, and regulation of methionine metabolism and ion uptake/excretion.

Plant membrane proteins are known to play critical roles in salt stress signaling and adaptation (Gilmore and Washburn 2010; Vertommen et al. 2011). In order to identify significantly

changed membrane proteins and determine their possible relevance to salt tolerance, the changes in membrane proteome of the M14 plants leaves in response to salt stress (0, 200, 400 mM NaCl) using an iTRAQ LC-MS/MS technology for quantitative proteomic analysis were reported (Li et al. 2015). Fifty membrane proteins exhibiting differential protein level changes were identified, which were mainly involved in transport, metabolism, protein synthesis, photosynthesis, protein folding and degradation, signal transduction, stress and defense, energy metabolism, and cell structure. This study provided the membrane adaptive mechanisms in sugar beet M14 under long-term (7 days) and moderate salt stress (200 mM, 400 mM NaCl) at the protein level.

Protein phosphorylation is one of the most common and important post-translational modifications (PTMs), which plays important roles in the regulation of diverse processes including metabolism, transcription/translation, protein degradation, homeostasis, cellular communication/signaling, proliferation, differentiation, and cell survival (Hunter 2000; Thingholm et al. 2009). Plants respond to salt stress by triggering phosphorylation cascades (Gong et al. 2004). In order to identify significantly changed proteins and phosphoproteins and determine their potential relevance to salt stress response, the characteristics of the M14 plants leaves at time points 30 min and 60 min after 0, 200, and 400 mM NaCl treatment using label-free quantitative proteomics approaches were reported (Yu et al. 2016). One hundred fourteen differentially expressed proteins were identified under salt stress. One hundred eighty nine phosphoproteins exhibited significant changes at the phosphorylation level under salt stress. The salt stress responsive proteins and phosphoproteins were mainly involved in signal transduction and kinase, transport, photosynthesis, metabolism, protein folding and degradation, and stress and defense. Exciting findings from this quantitative phosphoproteomics study include: (1) Several signaling components kinases under short term salt stress were found, e.g. 14-3-3 and mitogen-activated protein kinases (MAPK) and calcium-dependent protein kinases (CDPK); (2) Protein phosphorylation events were not limited to signal transduction spreading across key physiological processes including transport, transcription, metabolism, and stress and defense. This explained how the M14 plants can quickly respond to salt stress, make adaptive changes in cellular biochemical and physiological processes, and achieve tolerance for long-term growth and development. This study revealed the salt responsive mechanisms of the special sugar beet M14 line under short-term (30 min and 60 min) and moderate salt stress (200 mM, 400 mM NaCl) using label-free quantitative phosphoproteomics.

Acetylation of histone proteins is a type of chromatin modification that facilitates the activation of genes in plant defense against a variety of environmental stresses (Kim et al. 2015; Hu et al. 2011). Deciphering the exact mechanisms of chromatin modifications under abiotic stress conditions is important for improving crop plants' performance and yield. The salt stress responses of *B. v. vulgaris* and *B. v. maritima* were compared (Yolcu et al. 2016). The chromatin immunoprecipitation (ChIP) assay in control and salt stressed (250 and 500 mM NaCl) plants were performed and the enrichment of acetylation in the associated chromatin sites was compared. The transcriptional activation of one POD encoding gene that was associated with the elevated levels of acetylation in H3K9 and H3K27 sites was found. The acetylation patterns were remarkably different between two subspecies in which the highest acetylation levels were found at H3K9 and H3K27 in wild beet and sugar beet, respectively.

10.5.2 The Proteome Analysis of Sugar Beet in Response to Other Stresses

To investigate how the sugar beet was affected by K^+ deficiency and by substitution of K^+ by Na^+, the comparative proteomic approach 2-DE was applied to obtain 27 significant changes protein among the treatments (Pi et al. 2016). Based

on the proteomic results, K^+ was assumed to engage in a wide range of metabolic pathways, especially photosynthesis and cellular respiration. It was found that Na^+ may stimulate photosynthesis and restrict cellular respiration in K^+ deficiency. Na^+ was therefore in some ways able to recover damage due to K^+ deficiency at the protein level, but cannot functionally replace K^+ as an essential nutrient.

Iron is the fourth most abundant element in the Earth's crust and is an essential micronutrient for all living organisms including plants. However, its low availability in neutral or alkaline soils, which account for approximately 30% of the world's arable soils, causes Fe deficiency (Abadía et al. 2004). Iron deficiency is the most common nutritional disorder in many plants and typical symptoms include chlorosis of young leaves (leaf yellowing) and reduced crop yield and quality (Abadía et al. 2011). In order to understand the Fe deficiency mechanisms of sugar beet, the protein profile changes of the sugar beet apoplastic fluid induced by Fe deficiency and Fe resupply to Fe-deficient plants were studied by 2-DE (Ceballos-Laita et al. 2015). One hundred nine unique proteins were identified, 75% of which were classified into stress and defense, protein metabolism, cell wall and carbon metabolism. The result showed that protein homeostasis in the leaf apoplast fluid was well-maintained upon Fe shortage. The identification of three chitinase isoforms among proteins increasing in relative abundance with Fe-deficiency suggested that one of the few effects of Fe deficiency in the leaf apoplast proteome included cell wall modifications. Iron resupply to Fe deficient plants changed the relative abundance of 16 spots when compared to either Fe-sufficient or Fe-deficient samples.

Plants grown under iron deficiency show different morphological, biochemical and physiological changes (Santi and Schmidt 2009; Jin et al. 2007). These changes include the elicitation of different strategies to improve the acquisition of Fe from the rhizosphere, the adjustment of Fe homeostasis processes and a reorganization of carbohydrate metabolism (Larbi et al. 2010; López-Millán et al. 2001). In order to provide a holistic view of the metabolic processes occurring in sugar beet plants under different Fe status, the changes induced in the root tip proteome and metabolome of sugar beet plants in response to Fe deficiency and resupply were characterized (Rellán-Alvarez et al. 2010). By 2-DE, iron deficiency resulted in changes in the relative amounts of 22 proteins. Metabolites in root tip extracts were analyzed by gas chromatography-MS, 26 identified metabolites changed significantly with Fe deficiency. Iron deficiency induced increases in the relative amounts of proteins and metabolites associated to glycolysis, tricarboxylic acid cycle (TCA) and anaerobic respiration. Furthermore, a protein not present in Fe-sufficient roots, dimethyl-8-ribityllumazine (DMRL) synthase, was present in high amounts in root tips from Fe-deficient sugar beet plants and gene transcript levels were higher in Fe-deficient root tips. The increases in DMRL synthase and in raffinose family of oligosaccharides (RFO) sugars were the major changes induced by Fe deficiency and resupply in root tips of sugar beet plants. This study confirmed the increase in proteins and metabolites related to carbohydrate metabolism and TCA cycle pathways.

Zinc is an essential micronutrient for plants and plays structural and/or catalytic roles in many essential physiological processes, being the only metal present in all enzyme classes and the second most abundant transition metal after Fe (Vallee and Auld 1990). However, when present at high concentrations, Zn can be toxic to plants (Foy et al. 1978; Singh and Agrawal 2007). To investigate comprehensively the effects of Zn toxicity in roots of sugar beet plants, changes induced by three levels of Zn in the root proteome from *B. vulgaris* were studied by 2-DE (Gutierrez-Carbonell et al. 2013). Three hundred twenty spots were consistently detected and 16, 16 and 35 of them showed significant changes in relative abundance as a result of the 50, 100 and 300 μM Zn treatments, respectively, when compared to the control (1.2 μM Zn). Forty-four spots had consistent changes between all treatments, and 41 differentially expressed proteins were identified. At low and mild Zn excess, Complex I of the mitochondrial transport chain and the oxidative phosphorylation were mildly impaired, and an effort to compensate

this effect by increasing glycolysis was observed. At high Zn excess, a general metabolism shutdown occurred, as denoted by decreases in the aerobic respiration and by an impairment of the defense systems against oxidative stress. This study suggested that metabolic changes at high Zn supply reflected cell death, while changes at low and mild Zn supplies may rather explain the metabolic reprogramming occurring upon Zn toxicity. Results also suggested that Zn competition with divalent ions including Fe may contribute to many Zn toxicity symptoms, especially at low and moderate Zn supplies.

10.5.3 The Tolerance and Adaptation Mechanisms in Translational Level Changes of Sugar Beet in Response to Biotic and Abiotic Stresses

Proteomics research in sugar beet was mainly focused on salt stress. Four hundred thirty eight differentially expressed proteins in sugar beet M14 involved in short-term and long-term salt stress responses and 9 additional differential expression proteins from *Beta vulgaris* L. cv. Evita under salt stress were identified and classified into different functions. Interestingly, 189 differentially expressed phosphoproteins at the phosphorylation level and two acetylated proteins at acetylation levels were found in sugar beet in response to salt stress. One hundred ninety nine differentially expressed proteins in sugar beet which participated in other abiotic stresses were identified and analyzed. Proteomics research in sugar beet provides a large number of stress-responsive proteins resources, which has a prospect to improve stress resistance of sugar beet and other crops by genetic engineering.

10.6 Conclusion

In this paper, we have reviewed the mechanisms of sugar beet responses to biotic and abiotic stresses at the physiological, gene functional, transcriptional and translational levels. For physiological changes, most research has been carried out for salt and drought stresses. It was concluded that the increase in the content of enzymes and non-enzymatic antioxidants were also the main strategies for sugar beet to regulate ROS and redox homeostasis under salt, drought and various stresses. The ability of keeping Na^+/K^+ ratio stable and cytosolic K^+ homeostasis were the main factors for sugar beet in response to salt stress. The increase in proline is one of the main physiological indicators for sugar beet in response to drought stress. The functions of genes from sugar beet in response to salt, cold and heavy metal stresses were mainly investigated by transgenic technologies. *BvPGDHa*, *BvSHMTa*, *BvCMO*, *BvNHX1*, *BvM14-glyoxalase* I, *BvM14-cystatin* and *BvM14-SAMS2* conferred salt tolerance for sugar beet; the transcriptional level of *BvpAPX* and the gene families of *BvSOD*, *BvPrX*, *BvAPX*, *BvPTOX* and *BvRBOH* were induced by salt stress. *BvIMP* was involved in cold tolerance for sugar beet. Transgenic sugar beets carrying the *StGCS-GS* gene from *Streptococcus thermophiles* accumulated more Cd, Zn and Cu ions in shoots than wild-type. The roles of *StGCS-GS* in enhancing Cd, Zn and Cu tolerance and accumulation in transgenic sugar beet may represent a highly promising new tool for phytoremediation. At the transcriptional level, the transcriptome analysis of sugar beet in response to salt, cold and biotic stresses were conducted by RNAseq or SSH methods. At the level of translation, more than 800 differentially expressed proteins in response to salt, K^+/Na^+ ratio, Fe deficiency, Fe resupply and heavy metal Zn conditions were identified by quantitative proteomics techniques, which were mainly involved in multiple metabolic pathways including photosynthesis, metabolism, protein folding and degradation, transport, stress and defense and other pathways. These studies have provided new insights unveiling the mechanisms of sugar beet in response to biotic and abiotic stresses.

Acknowledgements This research was supported by the National Science Foundation of China (Project 31501359: Study on phosphorylation sites of a Ser/Thr protein kinase from sugarbeet M14 line in response to salt stress, and

Project 31671751: Regulatory mechanisms of BvM14-glyoxalase I transcription factor in the response of sugar beet to salt stress). This research was also supported by the National Science Foundation of Heilongjiang (Project C2017057: Phosphoproteomics analysis of sugar beet M14 roots under salt stress), by China Postdoctoral Science Foundation (Project 204968: Identification of BvM14-STPK interacting proteins from sugar beet M14 and study on their salt tolerance function) and also by the Foundations (2014TD004, YJSCX2019-201HLJU). This work was supported by Heilongjiang Provincial Key Laboratory of Plant Genetic Engineering and Biological Fermentation Engineering for Cold Region. I.G. acknowledges support from Russian Ministry of Science and Higher Education (Project AAAA-A17-117020210023-1).

References

Abadía J, Álvarez-Fernández A, Rombolá AD, Sanz M, Tagliavini M, Abadía A (2004) Technologies for the diagnosis and remediation of Fe deficiency. Soil Sci Plant Nutr 50:965–971

Abadía J, Vázquez S, Rellán-Álvarez R, El-Jendoubi H, Abadía A, Alvarez-Fernández A, López-Millán AF (2011) Towards a knowledge-based correction of iron chlorosis. Plant Physiol Biochem 49:471–482

Adler G, Blumwald E, Bar-Zvi D (2010) The sugar beet gene encoding the sodium/proton exchanger 1 (*BvNHX*1) is regulated by a MYB transcription factor. Planta 232:187–195

Anwar A, Liu Y, Dong R, Bai L, Yu X, Li Y (2018) The physiological and molecular mechanism of brassinosteroid in response to stress: a review. Biol Res 51:46

Bagatta M, Pacifico D, Mandolino G (2008) Evaluation of the osmotic adjustment response within the genus *Beta*. J Sugar Beet Res 45:119–133

Bibikova TN, Jacob T, Dahse I, Gilroy S (1998) Localized changes in apoplastic and cytoplasmic pH are associated with root hair development in *Arabidopsis thaliana*. Development 125:2925–2934

Bilova T, Paudel G, Shilyaev N, Schmidt R, Brauch D, Tarakhovskaya E, Milrud S, Smolikova G, Tissier A, Vogt T, Sinz A, Brandt W, Birkemeyer C, Wessjohann LA, Frolov A (2017) Global proteomic analysis of advanced glycation end products in the Arabidopsis proteome provides evidence for age-related glycation hot spots. J Biol Chem 292:15758–15776

Blumwald E, Poole RJ (1987) Salt tolerance in suspension cultures of sugar beet-induction of Na^+/H^+ antiport activity at the tonoplast by growth in salt. Plant Physiol 83:884–887

Ceballos-Laita L, Gutierrez-Carbonell E, Lattanzio G, Vázquez S, Contreras-Moreira B, Abadía A, Abadía J, López-Millán AF (2015) Protein profile of Beta vulgaris leaf apoplastic fluid and changes induced by Fe deficiency and Fe resupply. Front Plant Sci 6:145

Clifford T, Howatson G, West DJ, Stevenson EJ (2015) The potential benefits of red beetroot supplementation in health and disease. Nutrients 7:2801–2822

Cosgrove DJ (1997) Relaxation in a high-stress environment: the molecular bases of extensible walls and cell enlargement. Plant Cell 9:1031–1041

Dohm JC, Minoche AE, Holtgräwe D, Capella-Gutiérrez S, Zakrzewski F, Tafer H, Rupp O, Sörensen TR, Stracke R, Reinhardt R, Goesmann A, Kraft T, Schulz B, Stadler PF, Schmidt T, Gabaldón T, Lehrach H, Weisshaar B, Himmelbauer H (2014) The genome of the recently domesticated crop plant sugar beet (*Beta vulgaris*). Nature 505:546–549

Dunajska OK, Skorupa KM, Kurnik K, Tretyn A, Tyburski J (2014) Cloning and expression analysis of a gene encoding for ascorbate peroxidase and responsive to salt stress in beet (*Beta vulgaris*). Plant Mol Biol Report 32:162–175

Fénart S, Arnaud JF, De Cauwer I, Cuguen J (2008) Nuclear and cytoplasmic genetic diversity in weed beet and sugar beet accessions compared to wild relatives: new insights into the genetic relationships within the *Beta vulgaris* complex species. Theor Appl Genet 116:1063–1077

Fortmeier R, Schubert S (1995) Salt tolerance of maize (*Zea mays* L.): the role of sodium exclusion. Plant Cell Environ 18:1041–1047

Foy CD, Chaney RL, White MC (1978) The physiology of metal toxicity in plants. Ann Rev Plant Physiol 29:511–566

Foyer CH, Descourvières P, Kunert KJ (1994) Protection against oxygen radicals: an important defence mechanism studied in transgenic plants. Plant Cell Environ 17:507–523

Foyer CH, Bloom AJ, Queval G, Noctor G (2009) Photorespiratory metabolism: genes, mutants, energetics, and redox signaling. Annu Rev Plant Biol 60:455–484

Georgiev VG, Weber J, Kneschke EM, Denev PN, Bley T, Pavlov AI (2010) Antioxidant activity and phenolic content of betalain extracts from intact plants and hairy root cultures of the red beetroot *Beta vulgaris* cv. Detroit dark red. Plant Foods Hum Nutr 65:105–111

Gezginci-Oktayoglu S, Sacan O, Bolkent S, Ipci Y, Kabasakal L, Sener G, Yanardag R (2014) Chard (Beta vulgaris L. var. cicla) extract ameliorates hyperglycemia by increasing GLUT2 through Akt2 and antioxidant defense in the liver of rats. Acta Histochem 116:32–39

Ghosh A, Pareek A, Sopory SK, Singla-Pareek SL (2014) A glutathione responsive rice glyoxalase II, OsGLYII-2, functions in salinity adaptation by maintaining better photosynthesis efficiency and antioxidant pool. Plant J 80:93–105

Gilmore JM, Washburn MP (2010) Advances in shotgun proteomics and the analysis of membrane proteomes. J Proteome 73:2078–2091

Gong D, Guo Y, Schumaker KS, Zhu JK (2004) The SOS3 family of calcium sensors and SOS2 family of protein kinases in Arabidopsis. Plant Physiol 134:919–926

Guadagno CR, Greca MD, Santo AVD, D'Ambrosio N (2013) Nmr (^{1}h) analysis of crude extracts detects light stress in *Beta vulgaris* and *Spinacia oleracea* leaves. Photosynth Res 115:115–122

Guo D, Wang G, Kang C, Jia S, Liu L (1994) Studies on the interspecific crossing of cultivated sugar beet and *Beta corolliflora* Zoss. China Sugar Beet 3:2–7

Guo J, Li CF, Liu L, Sang LM, Chen M, Xu Y, Gai ZJ, Wang YB (2016) Variation of rhizosphere environmental factors of sugarbeet seedlings under Na_2CO_3 stress and their correlation. J Appl Ecol 27:904–910

Gutierrez-Carbonell E, Lattanzio G, Sagardoy R, Rodríguez-Celma J, Ríos Ruiz JJ, Matros A, Abadía A, Abadía J, López-Millán AF (2013) Changes induced by zinc toxicity in the 2-DE protein profile of sugar beet roots. J Proteome 94:149–161

Hanson AD, Wise R (1982) Biosynthesis, translocation, and accumulation of betaine in sugar beet and its progenitors in relation to salinity. Plant Physiol 70:1191–1198

Hoque TS, Hossain MA, Mostofa MG, Burritt DJ, Fujita M, Tran LS (2016) Methylglyoxal: an emerging signaling molecule in plant abiotic stress responses and tolerance. Front Plant Sci 7:1–11

Hossain MS, Elsayed AI, Moore M, Dietz KJ (2017) Redox and reactive oxygen species network in acclimation for salinity tolerance in sugar beet. J Exp Bot 68:1283–1298

Hu Y, Zhang L, Zhao L, Li J, He S, Zhou K, Yang F, Huang M, Jiang L, Li L (2011) Trichostatin A selectively suppresses the cold-induced transcription of the *ZmDREB1* gene in maize. PLoS One 6:22132

Hunter T (2000) Signaling – 2000 and beyond. Cell 100:113–127

Imadi SR, Kazi AG, Ahanger MA, Gucel S, Ahmad P (2015) Plant transcriptomics and responses to environmental stress: an overview. J Genet 94:525–537

Jang SJ, Wi SJ, Choi YJ, An G, Park KY (2012) Increased polyamine biosynthesis enhances stress tolerance by preventing the accumulation of reactive oxygen species: T-DNA mutational analysis of Oryza sativa lysine decarboxylase-like protein 1. Mol Cells 34:251–262

Jansen R, Stibbe C (2007) Impact of plant breeding on the profitability of sugar beet production. Int Sugar J 109:227–233

Jha B, Gontia I, Hartmann A (2012) The roots of the halophyte Salicornia brachiata are a source of new halotolerant diazotrophic bacteria with plant growth-promoting potential. Plant Soil 356:265–277

Jian Kang Z (2016) Abiotic stress signaling and responses in plants. Cell 167:313–324

Jin CW, You GY, He YF, Tang C, Wu P, Zheng SJ (2007) Iron deficiency-induced secretion of phenolics facilitates the reutilization of root apoplastic iron in red clover. Plant Physiol 144:278–285

Kaur C, Tripathi AK, Nutan KK, Sharma S, Ghosh A, Tripathi JK, Pareek A, Singla-Pareek SL, Sopory SK (2017) A nuclear-localized rice glyoxalase I enzyme, OsGLYI-8, functions in the detoxification of methylglyoxal in the nucleus. Plant J 89:565–576

Kim JM, Sasaki T, Ueda M, Sako K, Seki M (2015) Chromatin changes in response to drought, salinity, heat and cold stresses in plants. Front Plant Sci 6:1–12

Kirchhoff M, Svirshchevskaya A, Hoffman C, Schechert A, Jung C, Kopisch-Obuch F (2012) High degree of genetic variation of winter hardiness in a panel of *Beta vulgaris* L. Crop Sci 52:179–188

Kito K, Tsutsumi K, Rai V, Theerawitaya C, Cha-Um S, Yamada-Kato N, Sakakibara S, Tanaka Y, Takabe T (2017) Isolation and functional characterization of 3-phosphoglycerate dehydrogenase involved in salt responses in sugar beer. Protoplasma 254:2305–2313

Klemens PA, Patzke K, Trentmann O, Poschet G, Büttner M, Schulz A, Marten I, Hedrich R, Neuhaus HE (2014) Overexpression of a proton coupled vacuolar glucose exporter impairs freezing tolerance and seed germination. New Phytol 202:188–197

Kollist H, Zandalinas SI, Sengupta S, Nuhkat M, Kangasjärvi J, Mittler R (2019) Rapid responses to abiotic stress: priming the landscape for the signal transduction network. Trends Plant Sci 24:25–37

Köllner TG, Lenk C, Zhao N, Seidl-Adams I, Gershenzon J, Chen F, Degenhardt J (2010) Herbivore-induced SABATH methyltransferases of maize that methylate anthranilic acid using S-adenosyl-L-methionine. Plant Physiol 153:1795–1807

Krajka-Kuźniak V, Szaefer H, Ignatowicz E, Adamska T, Baer-Dubowska W (2012) Beetroot juice protects against N-nitrosodiethylamine-induced liver injury in rats. Food Chem Toxicol 50:2027–2033

Ku YS, Sintaha M, Cheung MY, Lam HM (2018) Plant hormone signaling cross talks between biotic and abiotic stress responses. Int J Mol Sci 3206:1–35

Larbi A, Morales F, Abadía A, Abadía J (2010) Changes in iron and organic acid concentrations in xylem sap and apoplastic fluid of iron-deficient Beta vulgaris plants in response to iron resupply. J Plant Physiol 167:255–260

Li H, Smigocki AC (2018) Transcriptome analysis of sugar beet root maggot (Tetanops myopaeformis) genes modulated by the Beta vulgaris host. Insect Sci 25:222–234

Li H, Cao H, Wang Y, Pang Q, Ma C, Chen S (2009) Proteomic analysis of sugar beet apomictic monosomic addition line M14. J Proteome 73:297–308

Li H, Pan Y, Zhang Y, Wu C, Ma C, Yu B, Zhu N, Koh J, Chen S (2015) Salt stress response of membrane proteome of sugar beet monosomic addition line M14. J Proteome 127:18–33

Li YY, Geng QY, Fei C, Fan H (2016) Physiological responses of sugar beet (beta vulgaris) to drought stress during vegetative development period under drip irrigation. Chin J Appl Ecol 27:201–206

Liu D, An Z, Mao Z, Ma L, Lu Z (2015) Enhanced heavy metal tolerance and accumulation by transgenic sugar beets expressing *Streptococcus thermophilus* StGCS-GS in the presence of Cd, Zn and Cu alone or in combination. PLoS One 10:1–15

López-Millán A, Morales F, Gogorcena Y, Abadía A, Abadía J (2001) Iron resupply- mediated deactivation of Fe-deficiency stress responses in roots of sugar beet. Aust J Plant Physiol 28:171–180

Lukyanov SA, Rebrikov D, Buzdin AA (2007) Suppression subtractive hybridization. In: Buzdinand AA, Lukyanov SA (eds) Nucleic acids hybridization modern applications. Springer, Berlin, pp 53–84

Lv C, Wang Y, Wang LJ, Li D, Adhikari B (2013) Optimization of production yield and functional properties of pectin extracted from sugar beet pulp. Carbohydr Polym 95:233–240

Lv X, Jin Y, Wang Y (2018) De novo transcriptome assembly and identification of salt-responsive genes in sugar beet M14. Comput Biol Chem 75:1–10

Ma C, Wang Y, Gu D, Nan J, Chen S, Li H (2017) Overexpression of S-adenosyl-l methionine synthetase 2 from sugar beet M14 increased *Arabidopsis* tolerance to salt and oxidative stress. Int J Mol Sci 18:1–16

Moliterni VM, Paris R, Onofri C, Orrù L, Cattivelli L, Pacifico D, Avanzato C, Ferrarini A, Delledonne M, Mandolino G (2015) Early transcriptional changes in Beta vulgaris in response to low temperature. Planta 242:187–201

Munns R (1993) Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant Cell Environ 16:15–24

Mutasa-Go¨ttgens ES, Joshi A, Holmes HF, Hedden P, Go¨ttgens B (2012) A new RNASeq-based reference transcriptome for sugar beet and its application in transcriptome-scale analysis of vernalization and gibberellin responses. BMC Genomics 13:99

Nagel J, Culley LK, Lu Y, Liu E, Matthews PD, Stevens JF, Page JE (2008) EST analysis of hop glandular trichomes identifies an O-methyltransferase that catalyzes the biosynthesis of xanthohumol. Plant Cell 58:186–200

Pandey A, Mann M (2000) Proteomics to study genes and genomes. Nature 405:837–846

Panella L, Lewellen RT (2007) Broadening the genetic base of sugar beet: introgression from wild relatives. Euphytica 154:383–400

Percey WJ, Shabala L, Wu Q, Su N, Breadmore MC, Guijt RM, Bose J, Shabala S (2016) Potassium retention in leaf mesophyll as an element of salinity tissue tolerance in halophytes. Plant Physiol Biochem 109:346–354

Pi Z, Stevanato P, Sun F, Yang Y, Sun X, Zhao H, Geng G, Yu L (2016) Proteomic changes induced by potassium deficiency and potassium substitution by sodium in sugar beet. J Plant Res 129:527–538

Pidgeon JD, Ober ES, Qi A, Clark CJA, Royal A, Jaggard KW (2005) Using multienvironment sugar beet variety trials to screen for drought tolerance. Field Crop Res 65:268–279

Poschet G, Hannich B, Raab S, Jungkunz I, Klemens PA, Krueger S, Wic S, Neuhaus HE, B€uttner M (2011) A novel Arabidopsis vacuolar glucose exporter is involved in cellular sugar homeostasis and affects the composition of seed storage compounds. Plant Physiol 157:1664–1676

Pradedova EV, Nimaeva OD, Saliaev RK (2014) Effect of stress conditions on the activity and isozyme composition of peroxidase of vacuoles and tissue extract of red beet roots. Izv Akad Nauk Ser Biol 41:254–263

Puthoff DP, Smigocki AC (2007) Insect feeding-induced differential expression of *Beta vulgaris* root genes and their regulation by defense-associated signals. Plant Cell Rep 26:71–84

Rathinasabapathi B, Burnet M, Russell BL, Gage DA, Liao PC, Nye GJ, Scott P, Golbeck JH, Hanson AD (1997) Choline monooxygenase, an unusual iron-sulfur enzyme catalyzing the first step of glycine betaine synthesis in plants: prosthetic group characterization and cDNA cloning. Proc Natl Acad Sci 94:3454–3458

Rayle DL, Cleland RE (1992) The acid growth theory of auxin-induced cell elongation is alive and well. Plant Physiol 99:1271–1274

Reeves RD, Baker AJM (2000) Phytoremediation of toxic metals. In: Raskin I, Ensley BD (eds) Using plants to clean up the environment. Wiley, New York, pp 193–229

Rellán-Alvarez R, Andaluz S, Rodríguez-Celma J, Wohlgemuth G, Zocchi G, Alvarez-Fernández A, Fiehn O, López-Millán AF, Abadía J (2010) Changes in the proteomic and metabolic profiles of Beta vulgaris root tips in response to iron deficiency and resupply. BMC Plant Biol 10:120

Ribeiro IC, Pinheiro C, Ribeiro CM, Veloso MM, Simoes-Costa MC, Evaristo I, Paulo OS, Ricardo CP (2016) Genetic diversity and physiological performance of Portuguese wild beet (Beta vulgaris ssp. maritima) from three contrasting habitats. Front Plant Sci 7:1–14

Roje SS (2006) Adenosyl-L-methionine: beyond the universal methyl group donor. Phytochemistry 67:1686–1698

Rontein D, Basset G, Hanson AD (2002) Metabolic engineering of osmoprotectant accumulation in plants. Metab Eng 4:49–56

Ros R, Muñoz-Bertomeu J, Krueger S (2014) Serine in plants: biosynthesis, metabolism, and functions. Trends Plant Sci 19:564–569

Russell BL, Rathinasabapathi B, Hanson AD (1998) Osmotic stress induces expression of choline monooxygenase in sugar beet and amaranth. Plant Physiol 116:859–865

Sahebi M, Hanafi MM, Azizi P, Hakim A, Ashkani S, Abiri R (2015) Suppression subtractive hybridization versus next-generation sequencing in plant genetic engineering: challenges and perspectives. Mol Biotechnol 57:880–903

Santi S, Schmidt W (2009) Dissecting iron deficiency-induced proton extrusion in Arabidopsis roots. New Phytol 183:1072–1084

Sen A, Alikamanoglu S (2014) Characterization of drought-tolerant sugar beet mutants induced with gamma radiation using biochemical analysis and isozyme variations. J Sci Food Agric 94:367–372

Shameer K, Naika MBN, Shafi KM, Sowdhamini R (2018) Decoding systems biology of plant stress for sustainable agriculture development and optimized food production. Prog Biophys Mol Biol 30089:1–21

Silveira JAG, Carvalho FEL (2016) Proteomics, photosynthesis and salt resistance in crops: an integrative view. J Proteome 143:24–35

Singh RP, Agrawal M (2007) Effects of sewage sludge amendment on heavy metal accumulation and consequent responses of Beta vulgaris plants. Chemosphere 67:2229–2240

Skorupa M, Gołębiewski M, Domagalski K, Kurnik K, Abu Nahia K, Złoch M, Tretyn A, Tyburski J (2016) Transcriptomic profiling of the salt stress response in excised leaves of the halophyte *Beta vulgaris ssp. maritima*. Plant Sci 243:56–70

Sulakhiya K, Patel VK, Saxena R, Dashore J, Srivastava AK, Rathore M (2016) Effect of *Beta vulgaris* Linn. leaves extract on anxiety- and depressive-like behavior and oxidative stress in mice after acute restraint stress. Pharm Res 8:1–7

Sümer A, Zörb C, Yan F, Schubert S (2004) Evidence of Naþ toxicity for the vegetative growth of maize (*Zea mays* L.) during the first phase of salt stress. J Appl Bot 78:135–139

Tabuchi T, Okuda T, Takashima Y, Azuma T, Nanmori T, Yasuda T (2006) Transcriptional response of glycinebetaine-related genes to salt stress and light in leaf beet. Plant Biotechnol 23:317–320

Takabe T, Rai V, Hibino T (2006) Metabolic engineering of glycinebetaine. In: Rai AK, Takabe T (eds) Abiotic stress tolerance in plants. Springer, Berlin, pp 137–151

Thingholm TE, Jensen ON, Larsen MR (2009) Analytical strategies for phosphoproteomics. Proteomics 9:1451–1468

Timmusk S, Abd ED, Copolovici L, Tanilas T, Kännaste A, Behers L, Nevo E, Seisenbaeva G, Stenström E, Niinemets Ü (2014) Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles. PLoS One 9:e96086

Tiziani S, Schwartz SJ, Vodovotz Y (2006) Profiling of carotenoids in tomato juice by one- and two-dimensional NMR. J Agric Food Chem 54:6094–6100

Turesson H, Andersson M, Marttila S, Thulin I, Hofvander P (2014) Starch biosynthetic genes and enzymes are expressed and active in the absence of starch accumulation in sugar beet tap-root. BMC Plant Biol 14:104

Vallee BL, Auld DS (1990) Zinc coordination, function, and structure of zinc enzymes and other proteins. Biochemistry 29:5647–5659

Valli V, Gómez-Caravaca AM, Di Nunzio M, Danesi F, Caboni MF, Bordoni A (2012) Sugar cane, sugar beet and maize are not only essential food crops, but also important feedstock for bio-ethanol. J Agric Food Chem 60:12508–12515

Van der VC, Schneidereit J, Driscoll S, Turner J, Kunert K, Foyer CH (2003) Oryzacystatin I expression in transformed tobacco produces a conditional growth phenotype and enhances chilling tolerance. Plant Biotechnol J 1:101–112

Vastarelli P, Moschella A, Pacifico D, Mandolino G (2013) Water stress in Beta vulgaris: osmotic adjustment response and gene expression analysis in ssp. vulgaris and maritima. Am J Plant Sci 4:11–16

Vertommen A, Panis B, Swennen R, Carpentier S (2011) Challenges and solutions for the identification of membrane proteins in non-model plants. J Proteome 74:1165–1181

Voelckel C, Gruenheit N, Lockhart P (2017) Evolutionary transcriptomics and proteomics: insight into plant adaptation. Trends Plant Sci 22(6):462–471

Wakeel A, Sümer A, Hanstein S, Yan F, Schubert S (2011a) In vitro effect of different Na+/K+ ratios on plasma membrane H+ -ATPase activity in maize and sugar beet shoot. Plant Physiol Biochem 49:341–345

Wakeel A, Asif AR, Pitann B, Schubert S (2011b) Proteome analysis of sugar beet (*Beta vulgaris* L.) elucidates constitutive adaptation during the first phase of salt stress. J Plant Physiol 168:519–526

Wang X, Elling AA, Li X, Li N, Peng Z, He G, Sun H, Qi Y, Liu XS, Deng XW (2009) Genome-wide and organ-specific landscapes of epigenetic modifications and their relationshipsto mRNA and small RNA transcriptomes in maize. Plant Cell 21:1053–1069

Wang Y, Zhan Y, Wu C, Gong S, Zhu N, Chen S, Li H (2012) Cloning of a cystatin gene from sugar beet M14 that can enhance plant salt tolerance. Plant Sci 191-192:93–99

Wang Y, Stevanato P, Yu L, Zhao H, Sun X, Sun F, Li J, Geng G (2017) The physiological and metabolic changes in sugar beet seedlings under different levels of salt stress. J Plant Res 130:1079–1093

Xia T, Apse MP, Aharon GS, Blumwald E (2002) IdentiWcation and characterization of a NaCl-inducible vacuolar Na^+/H^+ antiporter in *Beta vulgaris*. Physiol Plant 116:206–212

Yamada N, Takahashi H, Kitou K, Sahashi K, Tamagake H, Tanaka Y, Takabe T (2015) Suppressed expression of choline monooxygenase in sugar beet on the accumulation of glycinebetaine. Plant Physiol Biochem 96:217–221

Yang L, Ma C, Wang L, Chen S, Li H (2012) Salt stress induced proteome and transcriptome changes in sugar beet monosomic addition line M14. J Plant Physiol 169(9):839–850

Yang L, Zhang Y, Zhu N, Koh J, Ma C, Pan Y, Yu B, Chen S, Li H (2013) Proteomic analysis of salt tolerance in sugar beet monosomic addition line M14. J Proteome Res 12:4931–4950

Yolcu S, Ozdemir F, Güler A, Bor M (2016) Histone acetylation influences the transcriptional activation of POX in *Beta vulgaris* L. and *Betamaritima* L. under salt stress. Plant Physiol Biochem 100:37–46

Yongxue Z, Jingdong N, Bing Y (2016) OMICS technologies and applications in sugar beet. Front Plant Sci 7:1–11

Yu B, Li J, Koh J, Dufresne C, Yang N, Qi S, Zhang Y, Ma C, Duong BV, Chen S, Li H (2016) Quantitative proteomics and phosphoproteomics of sugar beet monosomic addition line M14 in response to salt stress. J Proteome 143:286–297

Zhang X, Liu S, Takano T (2008) Two cysteine proteinase inhibitors from Arabidopsis thaliana, AtCYSa and AtCYSb, increasing the salt, drought, oxidation and cold tolerance. Plant Mol Biol 68:131–143

Zhao S, Zhou N, Zhao ZY, Zhang K, Wu GH, Tian CY (2016) Isolation of endophytic plant growth-promoting bacteria associated with the halophyte Salicornia europaea and evaluating their promoting activity under salt stress. Curr Microbiol 73:574–581

Zhou N, Zhao S, Tian CY (2017) Effect of halotolerant rhizobacteria isolated from halophytes on the growth of sugar beet (*Beta vulgaris* L.) under salt stress. FEMS Microbiol Lett 364:1–7