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Research Article

De novo transcriptome assembly and identification of salt-responsive genes in sugar beet M14

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ABSTRACT

Sugar beet (*Beta vulgaris*) is an important crop of sugar production in the world. 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. To estimate a broad spectrum of genes involved in the M14 salt tolerance will help elucidate the molecular mechanisms underlying salt stress. Comparative transcriptomics was performed to monitor genes differentially expressed in the leaf and root samples of the sugar beet M14 seedlings treated with 0, 200 and 400 mM NaCl, respectively. 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 reactive oxygen species (ROS) scavenging system exhibited significant differences in the leaves and roots. Our transcriptomics results have provided new insights into the salt-stress responses in the leaves and roots of sugar beet.

Abbreviations: 1,3-PGA, 1,3-phosphoglycerate; 3-PGA, 3-phosphoglycerate; ACC, aminocyclopropane-1-carboxylate; ACP, acyl carrier protein; Accase, acetyl-coenzyme A carboxylase carboxyl transferase; ADP, adenosine diphosphate; AdoHcyase, adenosylhomocysteinase; AdoMet, S-adenosylmethionine; AOX, alternative oxidase; APX, ascorbate peroxidase; ATP, adenosine triphosphate; BADH, betaine aldehyde dehydrogenase; BAH, betaine aldehyde; bHLH, basic/helix_loop_helix; BP, binding protein; CAB, chlorophyll a/b-binding protein; CAT, catalase; CaX, calcium exchanger protein; CaH, sodium/calcium exchanger protein; CES, cellulose synthase; CMO, choline monooxygenase; CoA, coenzyme A; Cytb6f, cytochromeb6f; CNGC, cyclic nucleotide-gated ion channel; DHA, dehydroascorbate; DHAP, dihydroxyacetone phosphate; DHAR, dehydroascorbate reductase; E4P, erythrose-4-phosphate; EF, elongation factor; EIF, eukaryotic initiation factor; F-6-P, fructose-6-phosphate; FabB, 3-ketoacyl-ACP synthase; FabH, 3-oxoacyl-ACP synthase; FabG, 3-oxoacyl-acyl-carrier- protein reductase; FAD, flavin adenine dinucleotide; FBPase, D-fructose 1,6-bisphosphatase; Fd, ferredoxin; FNR, ferredoxin–NADP reductase; FTP, guanosine triphosphate; G, glucose; G-1-P, glucose-1-phosphate; GADPH, glyceraldehyde-3- phosphate dehydrogenase; GAP, glycerate 3-phosphate; GB, glycine betaine; GOX, glycolate oxidase; GPAT, glucose-1-phosphate adenylytransferase; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione S-transferase; HMT, homocysteine S-methyltransferase; HSP, heat shock protein; HXK, hexokinase; IDH, isocitrate dehydrogenase; KOC, outward-rectifying potassium channel; LHC, light-harvesting complex; LPD, dihydrolipoyl dehydrogenase; MDA, monodehydroascorbate; MDHAR, monodehydroascorbate reductase; Met, methionine; MIP, major intrinsic protein; NAC, nascent polypeptide-associated complex; NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; NBP, nucleic acid binding protein; NDGPD, NADP-dependent glyceraldehydes-3-phosphate dehydrogenase; NCKX, sodium/potassium/calcium exchanger; NHX, Na^+/H^+ exchanger; OEC, oxygen evolving complex; OEE, oxygen evolving enhancer protein; PDC, pyruvate decarboxylase; PDI, protein disulfide isomerase; PDHE1, pyruvate dehydrogenase E1; PFP, pyrophosphate–fructose 6-phosphate 1-phosphotransferase; PG, polygalacturonase; PGA, 3-phosphoglycerate; PGK, phosphoglycerate kinase; Pi, inorganic phosphate; PIP, plasma membrane intrinsic protein; PPA-AT, prephenate aminotransferase; PPIase, peptidyl-prolyl *cis*-trans isomerase; PPR, pentatricopeptide repeat-containing protein; PRK, phosphoribulokinase; PrxR, peroxiredoxin; PS I, photosystem I; PS II, photosystem II; Q, quinine; QH2, reduced quinone; R5P, ribose-5-phosphate; RCA, ribulose-1,5-bisphosphate carboxylase/oxygenase activase; RRF, ribosome-recycling factor; RPI, ribulose-5-phosphate isomerase; Ru5P, ribulose-5-phosphate; RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose-1,5-bisphosphate; S7P, sedoheptulose-7-phosphate; SAMDC, S-adenosylmethionine decarboxylase proenzyme; SBP, sedoheptulose-1,7-bisphosphate; SBPase, sedoheptulose-1,7-bisphosphatase; SCoAL, succinic acid synthetase; SF, splicing factor; SOS1, salt overly sensitive 1B; SOD, superoxide dismutase; SPDSY, spermidine synthase; TCTP, translationally controlled tumor protein; TEF, translation elongation factor; TF, transcription factor; TIF, translation initiation factor; TIP, tonoplast intrinsic protein; TK, transketolase; TL, thylakoid lumen; TPI, triose-phosphate isomerase; Trx, thioredoxin; UDP, uridine diphosphate; UDPG, uridine diphosphate glucose; UDPGDH, UDP-glucose 6-dehydrogenase; VHA, vacuolar H^+-ATP synthase subunit B; Xyl, xylanase; Xu5P, xylulose-5-phosphate.

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1. Introduction

Soil salinity is one of the most significant environment stresses affecting plant growth, development, productivity, and geographical distribution (Allakhverdiev et al., 2000; Zhu, 2001). Approximately 20% of cultivated lands in the world is affected by soil salinization (Munns and Tester, 2008), and it is predicted to reach 50% in 2050 (Wang et al., 2008). Improving plant salt tolerance toward enhancing productivity and bioenergy in saline soil is a focus of plant biology research. Plants exposed to salinity generate serials of significant changes at biochemical, physiological and molecular levels. Salt imposes three major challenges to plants such as; ion imbalance, osmotic stress and oxidative damage which lead to growth retardation, wilting or death (Parida et al., 2004; Gupta and Huang, 2014; Roy et al., 2014). In response to salt stress, plants have employed sophisticated strategies including ion transport, and synthesis of compatible solutes (such as glycine betaine and proline) to maintain ion homeostasis as well as stress response and adaptation (Zhang et al., 2011; Magnan et al., 2008; Wang et al., 2009; Xu et al., 2009; Silva and Geros, 2009; Zhu, 2002). Although some salt-responsive genes involved in membrane transport, signal transduction, redox regulation and other processes have been identified (Zhang et al., 2008), salt tolerance in plants is controlled by sophisticated signaling and metabolic networks that are not fully understood. More and more efforts have been devoted to revealing the molecular mechanisms of plant salt tolerance. A better knowledge of global gene expression related to salt-tolerance at the transcriptional level will help reveal the underlying regulatory and metabolic mechanisms.

Powerful high-throughput sequencing technologies are now available to capture gene expression patterns, making it possible to systemically address important questions about how plants deal with salt stress (Schuster, 2008; Mardis, 2008; Bräutigam and Gowik, 2010). As a revolutionized tool for gene expression analysis at the transcriptional level, RNA sequencing based on the platforms Illumina GAIIx and HiSeq™2000 has shown great utility in transcriptome of model plants or species closely related to model plants (such as *Arabidopsis*, rice, soybean, etc.), where genome information and gene annotations are available (Wong et al., 2006; Rabello et al., 2008; Fan et al., 2013). Due to the high throughput and coverage, the RNAseq technology is also suitable for gene expression profiles in non-model organisms without reference genomes (Hiremath et al., 2011; Zahaf et al., 2012; Postnikova et al., 2013). This technology has also been widely used in comparative transcriptomics to identify differences in transcript abundance among different cultivars, organs, developmental stages and/or treatment conditions (Shi et al., 2011; Zenoni et al., 2010; Sun et al., 2013).

Sugar beet (*Beta vulgaris*) accounts for nearly 30% of the world's annual sugar production, which is also an important source for bioethanol and animal feed (Liu et al., 2008). Although the cultivated beet (*B. vulgaris*) is classified as a highly salt-tolerant crop, due to the high efficient osmotic adjustment, the species was also sensitive to high salt during germination and early seedling (Liu et al., 2008). Sugar beet monosomic addition line M14 is an interspecific progeny crossed between cultivated sugar beet *Beta vulgaris* L. and the wild species *B. corolliflora* Zoss., which contains an wild chromosome of *B. corolliflora* and the entire *B. vulgaris* genome (Li et al., 2009). Previous work has shown that the M14 line can tolerate 500 mM NaCl treatment for 7 days. Yang et al., 2012 showed 28 unique leaf proteins and 29 unique root proteins involved in metabolism, protein folding and degradation, and photosynthesis exhibited significant changes, and they are likely to play important roles in plant salt tolerance. Combined with the suppression subtractive hybridization (SSH) libraries generated from the roots and leaves treated with 500 mM salt, the datasets provided some important genes that showed significant changes at both transcriptional and translational levels (Yang et al., 2012). In addition, the salt-responsive proteins in the M14 seedlings with 200 mM and 400 mM NaCl were determined using quantitative 2D gel and isobaric tag for relative and absolute quantitation (iTRAQ) approaches. A total of 67 unique proteins in leaves, 22 unique proteins in roots with 2D gel, and 75 unique proteins in leaves, 43 unique proteins in roots with iTRAQ had been identified. These proteins were found to be involved in photosynthesis, energy, metabolism, protein folding and degradation, and stress and defense. For example, several Calvin cycle related proteins, RuBisCO large subunit-binding protein subunit beta, RuBisCO activase and chloroplastic triosephosphate isomerase, showed significant accumulation in response to NaCl. Furthermore, some stress and defense-related proteins were identified using iTRAQ methods. Ascorbate peroxidase (APX) and monodehydroascorbate reductase (MDAR) were increased in leaves by salt stress. APX and MDAR are known to play a crucial role in glutathione-ascorbate cycle, which is one of the most important antioxidant protection systems for removing H_2O_2 (Yang et al., 2013).

To improve our knowledge of sugar beet global gene expression profiles and identify the genes involved in the salt tolerance of M14, the HiSeq™ 2000 sequencing platform was used to generate a reference transcriptome dataset and explore differentially expressed genes (DEGs) for studying the salt tolerance mechanisms in the leaves and roots treated with 200 and 400 mM NaCl for 7 days, respectively. We compared these libraries of salt-treated and control samples to identify genes with significant transcriptional changes and verified the transcript changes through qRT-PCR. Meanwhile, transcription factors involving salt responses were annotated and classified. The expression profiles of genes involved in ROS scavenging were analyzed. These results provide a valuable genetic resource for further investigation of the molecular mechanisms underlying salt tolerance in sugar beet that may be translated to other agricultural crops.

2. Materials and methods

2.1. Plant growth and NaCl treatment

Sugar beet M14 line seeds were germinated and grown in a growth chamber with a 13 h light/11 h dark cycle, 25/20 °C day/night temperature, 450 μmol m^{-2} s^{-1} light intensity and a relative humidity of 70%. Salt treatment was initiated three weeks after sowing. Based on our previous studies, M14 growth was barely affected with 100 mM NaCl treatment, but inhibited when the NaCl concentration increased to 200 mM, and M14 seedlings started to show cell death at 500 mM NaCl for 7 days (Yang et al., 2012). Therefore, three NaCl concentrations were included in the experiment, 0 (control), 200 and 400 mM. In order to reduce plasmolysis caused by the osmotic shock (Shavrukov, 2013), NaCl was gradually increased at a rate of 50 mM each day until the desired concentration was reached (Sanchez et al., 2008). The Hoagland nutrient solution was replaced daily to maintain a stable NaCl concentration.

2.2. Sample collection, fresh weight, dry weight and membrane permeability of M14 seedlings

For each treatment, 9 seedlings (three biological replicates) from the control and treated M14 seedlings were harvested after 7 day salt treatment for RNA sequencing or other physiological measurement. The fresh weight (FW) was calculated after harvest and dry weight (DW) was measured after drying for 3 days at 80 °C. About 5 g fresh tissues were prepared for analyzing membrane permeability (Lutts et al., 1995).