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Research article

Proteomic profiling sheds light on alkali tolerance of common wheat (*Triticum aestivum* L.)

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ABSTRACT

Alkali (high-pH) stress is an important factor limiting agricultural production and has complex effects on plant metabolism. Transcriptomics is widely used in the discovery of stress-response genes, but it provides only a rough estimation for gene expression. Proteomics may be more helpful than transcriptomics for the discovery and identification of stress-response genes. In this study, wheat plants were treated with sodic alkaline stress (50 mM, $NaHCO_3$: Na_2CO_3 = 1:1; pH 9.7), and then proteomic analysis was carried out on control and stressed plants. We detected 3,104 proteins, including 69 alkaline stress-response proteins. Five superoxide dismutases, three malate dehydrogenases, three dehydrin proteins, and one V-ATPase protein were upregulated in sodic alkaline-stressed wheat roots. We propose that these salinity response proteins may be important for ion homeostasis and osmotic regulation of sodic alkaline-stressed wheat. Additionally, two malic enzymes and many enzymes involved in the tricarboxylic acid cycle (TCA) were downregulated in the roots. The upregulation of malate dehydrogenase and the downregulation of TCA enzymes and malic enzymes may enhance the accumulation of malate in sodic alkaline-stressed wheat roots. Previous studies have demonstrated that the accumulation of malate in roots is a crucial adaptive mechanism of wheat to sodic alkaline stress. Herein, our proteomics results provided molecular insights into this adaptive mechanism.

1. Introduction

Soil salt stress and alkali (high-pH) stress severely affect agricultural productivity (Läuchli and Lüttge, 2002). Usually, salt-alkalinized soils contain various ions, such as K^+, Na^+, Ca^{2+}, Mg^{2+}, NO_3^-, Cl^-, SO_4^{2-}, HCO_3^-, and CO_3^{2-} (Läuchli and Lüttge, 2002). NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3 are the main salts that limit plant growth in these salt-alkalinized soils (Kawanabe and Zhu, 1991). It had been documented that the damaging effect of alkaline salts ($NaHCO_3$ and Na_2CO_3) to plants is more severe than that of neutral salts (NaCl and Na_2SO_4) of the same salinity (Yang et al., 2008, 2009). In many saline areas, soils contain both neutral (NaCl and Na_2SO_4) and alkaline salts ($NaHCO_3$ and Na_2CO_3), leading to a combination of salt stress and alkali stress, which frequently occur simultaneously. In northeastern China, for example, 70% of grassland soil is alkalinized, a figure which is still expanding (Kawanabe and Zhu, 1991). Only a few alkali-tolerant plants can survive in these highly alkalinized grasslands and croplands. Salt tolerance has been a popular research topic for the past forty years, but few studies have focused on the alkali tolerance of plants. Understanding mechanisms of alkali tolerance in plants will be important for exploiting and recovering alkalinized soil. Although several researchers have reported some interesting information on alkali tolerance in transgenic plants (Wang et al., 2016; He et al., 2017), as well as the association between alkali tolerance and organic acid metabolism (Ma et al., 2017), gene expression (Zhang et al., 2013; Meng et al., 2017), metabolic profiling (Guo et al., 2016), and proteomic profiling (Yu et al., 2013; Zhang et al., 2016), alkali-tolerance mechanisms remain poorly understood.

Abbreviations: TCA, Tricarboxylic Acid Cycle; NHX, sodium/hydrogen exchange protein; CAT, catalase; GST, glutathione S-transferase; SOD, superoxide dismutase; GSH, glutathione synthase function; P5CS, pyrroline-5-carboxylate synthetase functions; PP2C, protein phosphatase 2C; LEA, late embryogenesis abundant protein; DHN, dehydrin; AMT, NH_4^+ uptake transporter; NRT, NO_3^- transporter; SnRK2, serine/threonine-protein kinase; *GS*, glutamine synthetase gene; *GOGAT*, glutamate synthase; ABI5, ABA-insensitive 5; OA, Organic acid

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Transcriptomics is the most widely used strategy by which to estimate the global expressional status of genes in cells, tissues, and organisms. Transcriptomics provides only a rough estimation of the expression of each specific gene because an mRNA with high abundance may be degraded rapidly or be inefficiently translated, producing little protein (Gygi et al., 1999). Additionally, post-translational modifications are necessary for the functions of many proteins. Proteomics is more complicated than transcriptomics, yet can directly confirm the presence of a specific protein and measure its concentration and modifications (Gstaiger and Aebersold, 2009; Bensimon et al., 2012; Kumar et al., 2016). Particularly, proteomics may be more helpful than transcriptomics for the discovery and identification of stress-response genes (Gstaiger and Aebersold, 2009; Bensimon et al., 2012; Kumar et al., 2016).

Wheat is an important source of human food. As wheat has wider adaptabilities to different stressful environments than any other food crop, it is grown on a greater proportion of the land area (Dubcovsky and Dvorak, 2007). Understanding the stress-tolerance mechanisms operating in wheat has practical significance for improving the stress tolerance of this crop. Although a large amount of transcriptomics research has focused on the salinity stress tolerance of wheat, few researchers have applied proteomics to investigate wheat salinity stress tolerance (Capriotti et al., 2014; Lv et al. 2016; Xu et al., 2016; Jiang et al., 2017). These findings showed that differentially accumulated proteins under salinity stress were involved in protein metabolism (Capriotti et al., 2014), energy production (Capriotti et al., 2014), antioxidant enzymes (Lv et al., 2016; Jiang et al., 2017), linolenic acid metabolism (Xu et al., 2016), photosynthesis (Xu et al., 2016), and ion transport (Jiang et al., 2017). A limited number of proteomics researchers have focused on alkali stress tolerance of plants (Yu et al., 2013; Pang et al., 2016; Zhang et al., 2016; Long et al., 2019), and no proteomic research on alkali tolerance in wheat has been reported. In the present study, wheat plants were exposed to sodic alkaline stress (50 mM, 1:1 M ratio of $NaHCO_3$ to Na_2CO_3) for 2 days, followed by characterization of the differentially expressed proteins of the control and sodic alkaline treatment plants. These proteomics data will provide insights into the mechanisms of alkali tolerance in wheat, as well as permit molecular dissection of the physiological responses of wheat to alkali stress previously reported (Yang et al., 2007, 2008, 2010; Guo et al., 2015, 2017).

2. Materials and methods

2.1. Plant materials and stress treatment

'Bobwhite', a common wheat (*Triticum aestivum* L.) cultivar, was chosen as the experimental plant material because it is widely used in transgenics and molecular investigations. Seeds of Bobwhite were sown in 17-cm diameter plastic pots containing washed sand. Each pot contained five seeds, and the pots were watered with half-strength Hoagland nutrient solution daily. All pots were incubated in the greenhouse (25 ± 1.5 °C during the day and 19 ± 1.5 °C during the night). From when the seedlings were 2-weeks-old, sodic alkaline stress treatments were performed every day by the application of nutrient solution containing the appropriate salts. Two alkaline salts were mixed in a 1:1 M ratio ($NaHCO_3$ to Na_2CO_3, pH 9.7) as the sodic alkaline stress treatment, and total salinity was 50 mM. Control plants were maintained by watering with half-strength Hoagland nutrient solution. The treatment duration was 2 days. Leaves or roots of five individual seedlings from one pot for each treatment were pooled to form a biological replicate, with four biological replicates for each treatment.

2.2. Protein extraction and alkylation

Protein extraction and alkylation were conducted according to the following protocols:

1. A sample (0.4 g) of fresh leaves or roots were ground in liquid nitrogen, and then 5 mL trichloroacetic acid/acetone solution (10% trichloroacetic acid in acetone) were added onto the ground tissue to extract the total protein.
2. The extract was incubated in the dark at −20 °C for 3 h. After centrifugation (at 13000 × *g*, 4 °C for 30 min), the supernatant was discarded and 1 mL acetone was applied to wash the pellet containing the proteins.
3. A volume of 1.5 mL buffer A (8 M urea, 4% CHAPS, 30 mM HEPES, 2 mM Na_2EDTA, 10 mM dithiothreitol (DTT), and 1 mM phenylmethylsulfonyl fluoride (PMSF); pH 8.2) was used to resuspend the pellet.
4. After centrifugation (at 13000 × *g*, 4 °C for 30 min), 15.8 μL 200 mM DTT was added to 0.3 mL of the supernatant from step 3. After incubation at 56 °C for 1 h, 35 μL 1 M iodoacetamide (IAM) was rapidly added to the solution, and then proteins in the sample were precipitated by adding 1 mL cold acetone.
5. Finally, 700 μL 50 mM NH_4HCO_3 was added to suspend the pellet from step 4. The protein samples were quantified using Bradford method (Bradford, 1976).

2.3. Protein digestion and peptide purification

We used 1 μg trypsin to digest 40 μg protein into peptides by incubating at 37 °C for 16 h, followed by the application of the digestate to C_{18} Pipette Tips (product ID 87784; Thermo Fisher Scientific, Fresno, CA, USA) to purify the peptides according to the manufacturer's instructions. Next, the purified peptides were loaded onto a LC-MS/MS system consisting of a nano-flow UHPLC System (UPLC EASY-nLC 1200) and a Q-Exactive mass spectrometer (Thermo Scientific, Germany).

2.4. LC-MS/MS analysis of the purified peptides

The peptides were loaded onto an Acclaim PepMapTM 100 column (C_{18}, 100 μm i.d. × 2 cm, 5 μm particle size, 100 Å) on the UPLC EASY-nLC 1200 system, before being separated on an Acclaim PepMap™ RSLC column (C_{18}, 50 μm × 15 cm, 2 μm, 100 Å) with a linear gradient of mobile phase A (0.1% formic acid) and mobile phase B (0.1% formic acid and 80% acetonitrile). Full MS scans were performed by the Q-Exactive mass spectrometer in the range of m/z 350–2000 at a resolution of 70,000. The AGC target value was set at 3e6 with maximum injection time of 50 ms. The 15 most abundant precursor ions were considered for fragmentation using higher-energy collisional-induced dissociation with a normalized collision energy of 27. The AGC target value of MS/MS scan was set at 1e5 and the resolution was 17,500. MS-MS data were generated by Xcalibur software (version 2.1; Thermo Scientific, USA).

2.5. Label-free quantification analysis

The MS-MS data were exposed to label-free proteomics analysis (Thermo Scientific, USA) according to the manufacturer's protocol. Protein searching and label-free quantification were performed on Proteome Discoverer software 2.2 (Thermo Scientific, USA) against the wheat reference genome (iwgsc_refseqv1.0). The proteins were identified using unique peptides with false detection rate (FDR) < 0.05. To minimize the effects of sample preparation processes on the quantification, peptide amounts for each protein were normalized to the total peptide amount of all the proteins in the sample. We quantified the protein content using the normalized abundances of the peptides, and differentially accumulated proteins (DAPs) under sodic alkaline and control conditions were discovered on the basis of significance using the *t*-test. DAP was defined as a fold-change > 2 and a P-value < 0.01 between the stress and control conditions.

2.6. Motif structure prediction

Motif structures of the proteins were predicted using MEMEsuit 5.0.1 (http://meme-suite.org/meme_5.0.1/tools/meme). The parameters were 10 motifs for searching and zero or one for site distribution.

2.7. qRT-PCR

Roots of five individual seedlings for each treatment were pooled to form a biological replicate, with four biological replicates for each treatment. Total RNA was isolated by TRIzol. The RNA samples were treated with DNaseI (Invitrogen), reverse-transcribed using SuperScriptTM RNase H-Reverse Transcriptase (Invitrogen), and then subjected to real-time PCR analysis using gene-specific primers (Table S1). Real-time quantitative PCR reactions were conducted using the StepOnePlus system and SYBR Green Real-time PCR Master Mix. *Actin* gene (KC775781.1) was used as internal control gene (Ravel et al., 2009; Yang et al., 2014). The primer sequences (5′-3′) of the *Actin* gene are CACACTGGTGTTATGGTAGG (forward) and AGAAGGTGTGATGC CAAAT (reverse). Gene expression data were normalized by $^{\triangle\triangle}$Ct method (Livak and Schmittgen, 2001).

2.8. Statistical analysis

Statistical analyses, in the form of the *t*-test and Pearson's correlation coefficient, were conducted using the statistical program SPSS, version 16.0 (IBM, Armonk, NY, USA). DAP was defined as a fold-change > 2 and a *P*-value < 0.01 between the stress and control conditions.

3. Results

3.1. Salinity tolerance proteins

In this work, we detected 3,104 proteins in the wheat plants (Table S2). Of the 3,104 expressed proteins, 41 proteins were involved in salinity tolerance (Table S3). These salinity-tolerance proteins mediate multiple salinity stress responses (Fig. 1A, Fig. 2 and Table 1):

(1) Eight V-ATPase proteins, one sodium/hydrogen exchange (NHX) protein and two H^+ pyrophosphatase proteins which mediate Na^+ compartmentalization.
(2) Five catalases (CATs), two glutathione S-transferases (GSTs), seven superoxide dismutases (SODs), and one glutathione synthase (GSH) function in quenching active free radicals to control oxidative stress.
(3) One pyrroline-5-carboxylate synthetase (P5CS) functions in synthesis of compatible solutes.
(4) Two K^+ channels function in K^+ uptake.
(5) Ten proteins related to the ABA-signaling system, including four protein phosphatase 2C (PP2C) proteins, three late-embryogenesis-abundant (LEA) proteins, and three dehydrin (DHN) proteins.

Most of these salinity-tolerance proteins were not upregulated by sodic alkaline stress (Fig. 1). For example, sodic alkaline stress did not change the abundance of NHX, but resulted in a decrease in the abundance of P5CS, four V-ATPase proteins, five CATs, and one H^+ pyrophosphatase protein, either in the leaves or in the roots (Fig. 1A). Additionally, under sodic alkaline stress, the abundance of two PP2C proteins decreased in the leaves, while one PP2C protein was downregulated in the roots (Fig. 1). Under sodic alkaline stress, only five Cu−Zn SODs, two GSTs, three DHNs and one V-ATPase were upregulated in the roots; however, two Cu−Zn SODs, one LEA protein, and one DHN were upregulated in the leaves (Fig. 1A).

3.2. Nitrogen and organic acid metabolism

Sodic alkaline stress greatly reduced the abundances of one NH_4^+ uptake transporter (AMT) and one NO_3^- transporter (NRT) in both leaves and roots. Similarly, five core N assimilation enzymes (two GDHs, two GSs, and one GOGAT) were downregulated in roots or leaves under sodic alkaline stress (Figs. 1A and 2). Three malate dehydrogenases were upregulated in the roots, and two malic enzymes were downregulated in the roots but not in the leaves. Many enzymes involved in the citric acid (TCA) cycle, such as isocitrate dehydrogenase, succinate dehydrogenase, citrate synthase, and succinate-CoA synthetase, were downregulated in either the roots or the leaves (Fig. 3).

3.3. Subgenome-preference of translation

As shown in Fig. 4A, in the roots, more A subgenome proteins were distributed in the −4 to 1 range of Log2 (fold change) than those of the B and D subgenome. Fig. 4B shows that, in the leaves, more A subgenome proteins were distributed in the −3 to 1 range of Log2 (fold change). The three subgenomes had similar protein numbers in the range of Log2 (fold change) < -3 and > 1. The wheat reference genome (iwgsc_refseqv1.0) used in the present work comprises 36,302 A-subgenome homeologous genes, 36,737 B-subgenome homeologous genes, and 35,021 D-subgenome homeologous genes. Although the three subgenomes consisted of almost equal number of genes, more proteins from the A subgenome were detected than from the B or D subgenomes under the present mass spectrometer conditions in both tissues under both conditions (Fig. 4B). Surprisingly, the percentage of A-subgenome protein number to total protein number was similar in control leaf (45.5%), control root (43.7%), stressed leaf (45.0%), and stressed roots (43.7%).

3.4. qRT-PCR

The results of label-free proteomic were validated by qRT-PCR (Table S1). The significant positive Pearson's correlation coefficient between the results of label-free analysis and qRT-PCR ($r = 0.660$, P value = 0.038) revealed that the fold-change values of label-free analysis were similar to those from qRT-PCR data (Table S1), and therefore that the results of label-free quantification analysis were reliable. Seven out of ten selected genes tested showed consistent results between qRT-PCR and label-free analysis (Table S1).

4. Discussion

4.1. Ion homeostasis and osmotic regulation

Salt stress involves osmotic stress and ion toxicity, and alkali stress exerts the same stress effects but with the additional high-pH stress. The high-pH surrounding the roots can directly cause Ca^{2+} and Mg^{2+} to precipitate, leading to nutrient deficiency (Wang et al., 2012). To cope with alkali stress, plants need to exclude Na^+ from or control Na^+ in the root, to compartmentalize Na^+ in organs or cells, to accumulate compatible solutes in cytoplasm, and to quench active free radicals in the cytoplasm and organelles. In the present work, we detected 41 proteins that function in the above regulatory processes (Fig. 1A). Generally, these salinity tolerance genes were upregulated under salinity stress (Munns and Tester, 2008; Plett and Møller, 2010). Surprisingly, most of these salinity-tolerance proteins were not upregulated in sodic alkaline stressed wheat plants (Fig. 1). Only five Cu−Zn SODs, three DHNs, and one LEA and one V-ATPase protein were upregulated in sodic alkaline stressed wheat plants. Consistent with the findings from our study, increasing abundance of V-ATPase protein under alkali stress was also observed in *Medicago* plants (Long et al., 2019) and *Puccinellia tenuiflora* (Yu et al., 2013), while several SOD proteins and a LEA protein were upregulated in alkali-stressed *Medicago* plants (Long

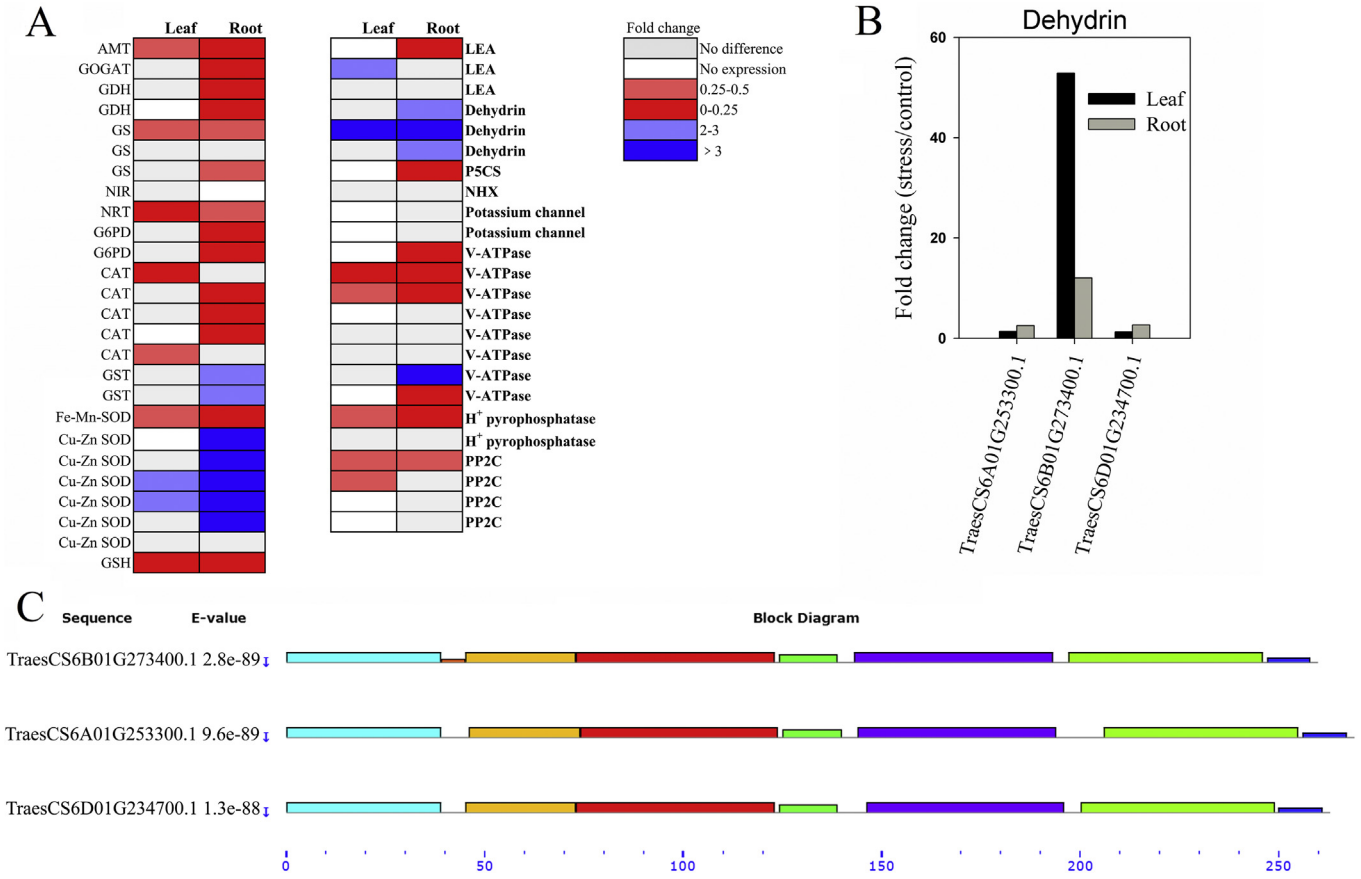

Fig. 1. Effects of sodic alkaline stress on protein abundances involved in salinity tolerance in wheat plants. (A) Heatmap of sodic alkaline tolerance proteins. Significant difference was defined as fold-change (stress/control) > 2 and *P* value < 0.01. The blue box represents upregulated proteins under sodic alkaline stress, and the red box represents downregulated proteins under sodic alkaline stress. (B) Abundances of three dehydrin (DHN) proteins derived from the A, B and D subgenomes. (C) Motif structures of the three DHN proteins derived from the A, B and D subgenomes. The motif structures were analyzed by MEMEsuit software. Wheat plants were treated with sodic alkaline stress ($NaHCO_3$: Na_2CO_3 = 1:1; pH 9.7) for two days. Each treatment and each tissue had four biological replicates, and each biological replicate comprised a pool of tissue from five plants. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

et al., 2019). We propose that the upregulated Cu−Zn SODs may play crucial roles in quenching active free radicals in sodic alkaline-stressed wheat plants, and the V-ATPase protein (TraesCS2A01G437500.1) may be important for Na^+ compartmentalization of wheat plants under sodic alkaline stress. As DHN and LEA proteins consist of tandem hydrophilic amino acids (with highly hydrophilic structures) (Goyal et al., 2005), they may have important roles in osmotic regulation and the prevention of protein aggregation during the response of wheat plants to sodic alkaline stress.

4.2. ABA-signaling system

The ABA-signaling system plays essential roles in the responses of plants to drought and salinity stresses (Vishwakarma et al., 2017). The ABA-signaling system comprises five core components: an ABA receptor (PYR/PYL), protein phosphatases 2C (PP2Cs), serine/threonine-protein kinase SRK2 (SnRK2), ABF/AREBs, and ABA-insensitive 5 (ABI5) (Vishwakarma et al., 2017). PP2C is a negative regulator of the ABA signaling system, and PYR–PP2C complex formation will lead to loss of PP2C function, thereby triggering down-stream signals (ABF and ABI5). ABF and ABI5 further modulate the multiple ABA-responsive genes such as *LEA* and *DHN* genes (Pedrosa et al., 2015; Sah et al., 2016). In the present study, under sodic alkaline stress, we observed several PP2C proteins that were downregulated, and one LEA protein and several DHN proteins that were upregulated (Fig. 1). The downregulation of the PP2C proteins indicated that sodic alkaline stress triggered the ABA-signaling system of wheat, and subsequently modulated expression of the down-steam ABA-responsive genes, such as the *LEA* and *DHN* genes. We propose that, during the response of wheat to sodic alkaline stress, the LEA and DHN proteins may exert roles through the ABA-signaling system.

4.3. Organic acid metabolism regulation

Previous work had shown that alkali stress can lead to the lack of anions through limiting uptake of NO_3^- or $H_2PO_4^-$ (Yang et al., 2007; Wang et al., 2012). For instance, alkali stress strongly limited nitrate uptake and assimilation by rice plants through decreasing expression of the glutamine synthetase (*GS*) gene and the glutamate synthase (*GOGAT*) gene (Wang et al., 2012). As in the rice plants, the sodic alkaline stress greatly decreased the abundance of key proteins that function in N uptake and assimilation of wheat plants (Fig. 1A), leading to limitation of N uptake by the roots. This may result in deficiency of NO_3^- in the wheat plant. Organic acids (OAs) play important roles in neutralizing cation excess (caused by deficiency of anions) and pH homeostasis (Yang et al., 2007, 2010). Previous publications had reported that, under alkali stress, some halophytes and glycophytes accumulate OAs in the shoots to balance cation excess, and also secrete OAs from their roots to reduce the high-pH conditions in the rhizosphere (Yang et al., 2007, 2010; Ding et al., 2018). Malate is one of the OAs most accumulated under alkali stress (Yang et al., 2007, 2010). Under alkali stress, several halophytes, rice and wheat significantly

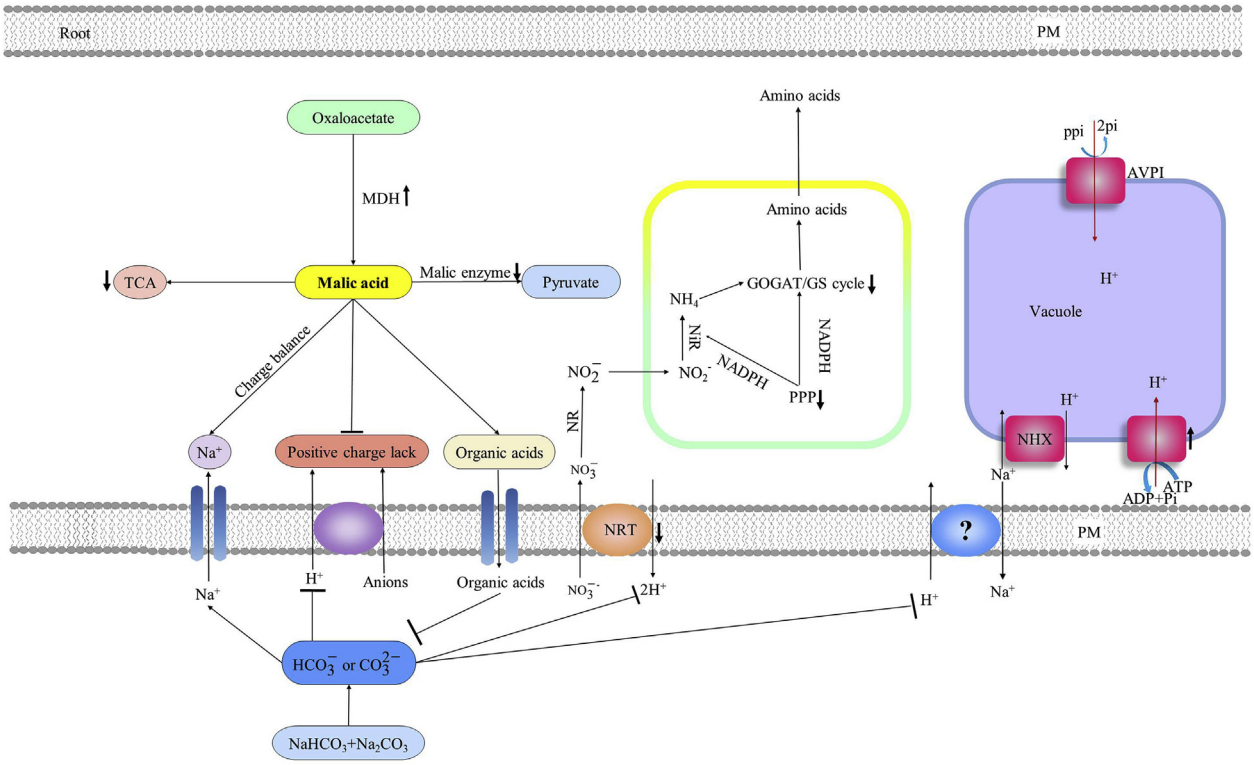

Fig. 2. Network of alkali tolerance of wheat plants on the basis of proteomic profiling. Wheat plants were treated with sodic alkaline stress ($NaHCO_3$: Na_2CO_3 = 1:1; pH 9.7) for two days. Each treatment and each tissue had four biological replicates, and each biological replicate was a pool of tissues from five plants. TCA, tricarboxylic acid cycle; AVP1, H^+ pyrophosphatase; MDH, malate dehydrogenase; GS, glutamine synthetase; GOGAT, glutamate synthase; NRT, nitrate transporter.

Table 1
General information of label-free proteomic analysis.

Identified protein number	
Genome	Number
A genome	1362
B genome	907
D genome	835
Total	3104
Detected peptide number	11960

Identified alkali tolerance protein			
Protein name	Number	Protein name	Number
G6PD	2	Aconitate hydratase	1
CAT	5	Isopropylmalate dehydratase	1
GST	2	Triosephosphate isomerase	1
SOD	7	Malate dehydrogenase	5
GSH	1	Malic enzyme	2
LEA	3	Isocitrate dehydrogenase	6
DHN	3	Succinate dehydrogenase	1
P5CS	1	Citrate synthase	1
NHX	1	Succinate-CoA synthetase	1
Potassium channel	2	AMT	1
V-ATPase	8	GOGAT	1
H^+ pyrophosphatase	2	GDH	2
PP2C	4	GS	3
		NIR	1
		NRT	1

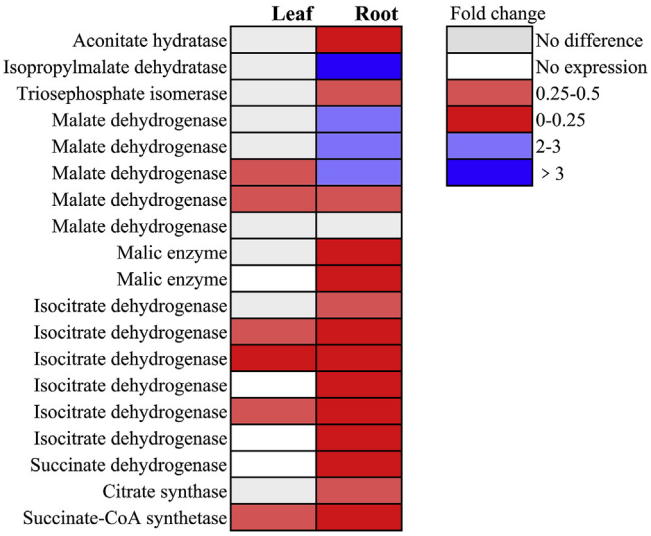

Fig. 3. Effects of sodic alkaline stress on protein abundances involved in organic acid metabolism in wheat plants. A significant difference was defined as a fold-change (stress/control) > 2 and a P value < 0.01. The blue box represents up-regulated proteins under sodic alkaline stress, and the red box represents down-regulated proteins under sodic alkaline stress. Wheat plants were exposed to sodic alkaline stress ($NaHCO_3$: Na_2CO_3 = 1:1; pH 9.7) for 2 days. Each treatment and each tissue had four biological replicates, with each biological replicate being a pool of the tissue from five plants. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

enhance the accumulation of malate, suggesting that accumulation of malate may be an alkali-tolerance strategy common to halophytes and glycophytes (Yang et al., 2007, 2008, 2010; Guo et al., 2015, 2017). Malate is a core component for many metabolic processes such as C_4 carbon fixation, the TCA cycle, and stomatal regulation. In C_3 plants such as wheat, malate is synthesized by malate dehydrogenase, and is degraded by malic enzymes or the TCA cycle (Fig. 2). Sodic alkaline stress strongly upregulated three malate dehydrogenases only in the roots, and downregulated two malic enzymes and many enzymes involved in the TCA cycle in the roots or the leaves (Fig. 3). The upregulation of the malate dehydrogenases and the downregulation of the

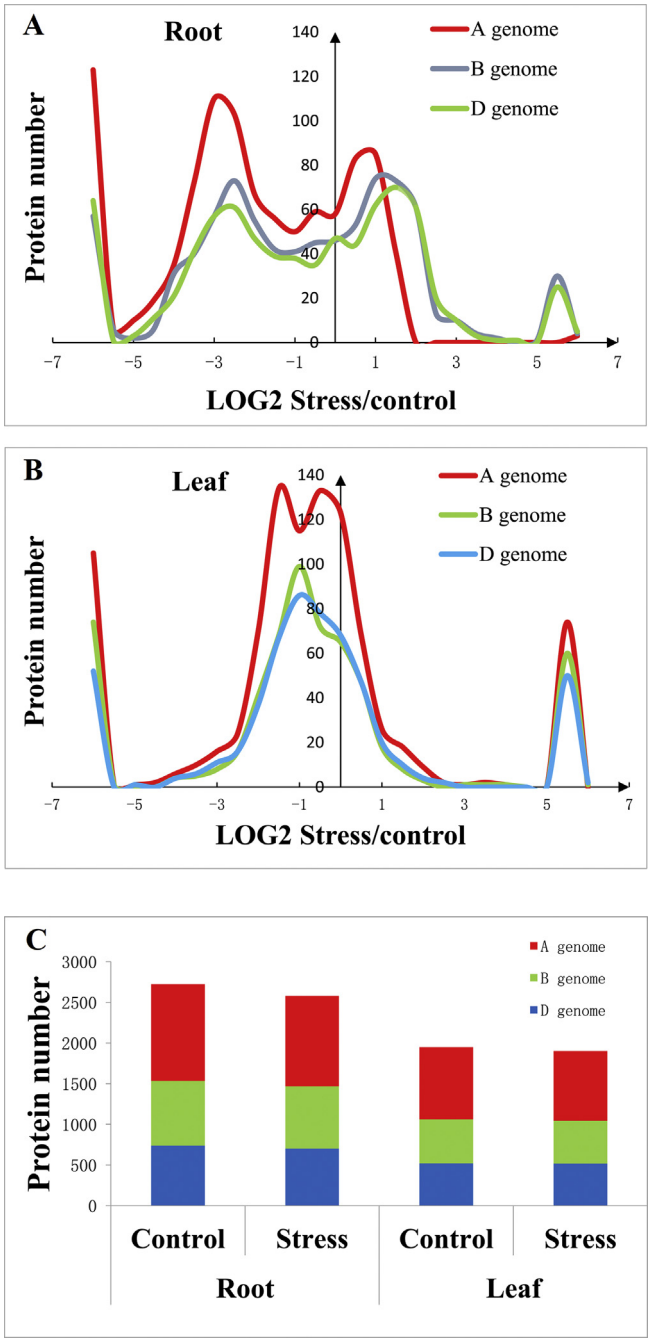

Fig. 4. Number of detected proteins from wheat plants under control and sodic alkaline stress conditions. The proteins derived from the three subgenomes (A, B and D) were identified through the use of Proteome Discoverer software 2.2 against the most recent wheat reference genome database (iwgsc_refseqv1.0) that includes 36,302 A-subgenome genes, 36,737 B-subgenome genes and 35,021 D-subgenome genes. (A) Frequency distribution curve of fold-change (stress/control) of protein abundances in wheat roots under sodic alkaline stress; (B) Frequency distribution curve of fold-change of protein abundances in wheat leaves under sodic alkaline stress; (C) Number of detected proteins derived from the three subgenomes (A, B and D) of wheat under control and sodic alkaline stress conditions.

TCA cycle enzymes and malic enzymes may promote the accumulation of malate in sodic alkaline-stressed wheat roots (Fig. 2). Thus, we have carried out a molecular dissection of the important physiological response of wheat to alkali stress (Guo et al., 2015, 2017), and propose that malate dehydrogenase and malic enzyme be regarded as candidate sodic alkaline-tolerance proteins.

4.4. Wheat revealed A-subgenome-preference for translation under both control and stress conditions

Accumulating archeological and molecular evidences has revealed that common wheat is a relatively young hexaploid species and has not yet exhaustively completed functional diploidization (Dubcovsky and Dvorak, 2007). The common wheat genome is comprised of A, B, and D subgenomes (Dubcovsky and Dvorak, 2007). Given interactions between the A, B, and D subgenomes, common wheat cultivars can display extensive homeolog-specific expression silencing and alternative splicing, resulting even in DNA fragment loss (Bottley et al., 2006; Akhunova et al., 2010). However, it is unclear whether three homeologs (A, B, and D) of a certain gene are all translated and, if so, how translation of the three homeologs is modulated. Although the three subgenomes consist of almost equal number of genes, we detected more proteins from the A subgenome in both tissues under both sodic alkaline stress and control conditions (Fig. 4), suggesting potential preference for the A subgenome with respect to translation in allohexaploid wheat (Fig. 4). We propose that the A subgenome may play more important roles in plant construction and stress responses of common wheat than do the B and D subgenomes. Additionally, we found that the three up-regulated DHNs have highly similar motif structures (Fig. 1C) yet derive from different subgenomes, which verifies that these three DHNs are homeologous proteins. Although all three DHNs were upregulated in the roots, the DHN of the B subgenome showed much greater up-regulation in both leaves and roots than did those of the A and D subgenomes (Fig. 1B). We propose that, under sodic alkaline stress, expression of the three DHN homeologs may be regulated through different mechanisms, such as experiencing different post-translational modifications, which should be investigated in the future.

5. Conclusions

Most of the salinity-tolerance proteins detected were not upregulated in sodic alkaline-stressed wheat plants. Three DHNs and several Cu—Zn SODs, a LEA protein and a V-ATPase protein were upregulated under sodic alkaline stress, and they may play important roles in the expression of alkali tolerance in wheat plants. The upregulation of malate dehydrogenase and the downregulation of TCA enzymes and malic enzymes may explain the accumulation of malate in sodic alkaline-stressed wheat roots. In summary, this study proposes some candidate alkali-tolerance proteins, as well as carrying out a molecular dissection of the crucial physiological response of wheat plants to alkali stress. In addition, we found that the A subgenome showed preference for translation in allohexaploid wheat in both tissues under both sodic alkaline stress and control conditions.

Author contribution statement

LH, CX, and CY - Conception and design, execution of experiment, analysis and interpretation of the data, drafting of the article, and critical revision of the article for important intellectual content.

MW, JL, BX, NAB, AK, HW1(Hao Wang), HW2 (Huan Wang) - Execution of experiment analysis and interpretation of the data.

Conflicts of interest

The authors have declared that no competing interests exist.

Contribution

In the present work, proteomics data provided the insights into alkali tolerance of wheat. We also found that wheat exhibited A-subgenome-preference for translation under both control and stress conditions.

Acknowledgments

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Appendix A. Supplementary data

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