Generation of new salt-tolerant wheat lines and transcriptomic exploration of the responsive genes to ethylene and salt stress

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Abstract
Wheat (Triticum aestivum L.) is one of the most important staple crops. Most of wheat varieties are sensitive to salt stress, which is a major limitation for wheat production. To develop salt-tolerant wheat varieties for sustainable grain production, we used ethylmethylsulfonate to mutagenize over 90,000 seeds of the wheat cultivar Luyuan502. A total of 2000 salt-tolerant lines were identified after screening the plants in a salinized field. We further analyzed ethylene sensitivity, salt related physiological changes, and preliminary crop yield of the selected plants. We found 11 salt-tolerant lines exhibiting ethylene insensitivity and high grain production. Transcriptome analysis revealed 3278 differently expressed genes (DEGs) in the selected mutants, including the ones encoding CABs, PERs/PODs, BGLUs, CYP707s, and ZEPs. Most of DEGs may be involved in photosynthesis, biosynthesis of secondary metabolites, cyanoamino acid metabolism, carotenoid biosynthesis, thiamine metabolism, and cutin, suberine and wax biosynthesis pathways. In addition, 9 novel ETHYLENE RESPONSE FACTORS (ERFs) were identified and analyzed in the mutants. These ERFs may play critical roles in ethylene response and salt tolerance. The mutant lines with decreased ethylene sensitivity exhibited enhanced salt tolerance, suggesting that ethylene sensitivity was closely related with salt tolerance.

Keywords Wheat · Salt tolerance · Ethylene sensitivity · Mutagenesis · Transcriptome · ERFs

Abbreviations
ABA Abscisic acid
ACC Aminocyclopropane-1-carboxylate
AUX Auxin
BGLU Beta-glucosidase
BR Brassinosteroid
CAB Chlorophyll a-b binding protein
CAT Catalase
CK Cytokinrin
CTR Constitutive triple response
CYP Cytochrome P450
DEG Differentially expressed gene
EBF EIN3 binding F-box
EIL EIN3-like
EIN Ethylene insensitive
EMS Ethylmethylsulfonate
ERF Ethylene response factor
ET Ethylene
ETO Ethylene overproducer
ETR Ethylene response
FDR False discovery rate
GA Gibberellin
GO Gene ontology
JA Jasmonate
KEGG Kyoto encyclopedia of genes and genomes
MDA Malonic dialdehyde
NCED 9-Cis-epoxycarotenoid dioxygenase
PER Peroxidase
POD Peroxidase
RABT Reference annotation based transcript
ROS Reactive oxygen species
SA Salicylic acid
SL Strigolactone
SOD Superoxide dismutase
THI Hydroxymethylpyrimidine
ZEP Zeaxanthin epoxidase

Qian Ma, Huajian Zhou and Xinying Sui have made equal contribution.

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Introduction

Bread wheat (*Triticum aestivum* L., genome AABBDD) is originated from Middle East nearly 10,000 years ago. The hexaploid wheat was basically generated by a hybridization between the domesticated allotetraploid wheat (*Triticum turgidum* L., genome BBAA) and the diploid goat grass (*Aegilops tauschii* L., genome DD) (Dubcovsky and Dvorak 2007; Huang et al. 2002). The polyploid and complex genetic background strongly improved wheat plants’ adaptability to diverse environment and accelerated its spread in the world (Dubcovsky and Dvorak 2007; Feldman et al. 2012). Today, bread wheat is one of the most important world crops with about 220 million ha planting area in the world, feeding one-third of the global population, and providing one-fifth of the global caloric needs and half of the global carbohydrate requirements (Breiman and Graur 1995; Gill et al. 2004).

Despite of their high adaptability to diverse environments, wheat production is often challenged by unfavorable environmental changes, of which salt stress caused dramatic wheat yield reduction and quality loss due to the moderate tolerance of the bread wheat to the stress (Munns and Tester 2008). To date, soil salinity has affected one-fifth of irrigated agricultural land in the world, and it was thought that salinized land would continue to expand due to the increasing population and deterioration of environment (Yamaguchi and Blumwald 2005).

It was reported that salt accumulation induced osmotic stress with high levels of reactive oxygen species (ROS), and the excess Na⁺ would cause damage of chloroplast, reduction of photosynthesis and respiration, and inhibition of plant growth and production (Hasegawa et al. 2000; Liu et al. 2019; Munns 2002; Wang et al. 2015). On the other hand, plants have evolved some responsive mechanisms to alleviate the negative effects. For example, the expressions of the genes encoding superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), can be activated to alleviate the ROS-induced oxidative damage in salt stress (Abogadallah 2010). The Na⁺ concentration can be kept to a low level by Na⁺ exclusion and compartmentalization, to protect the K⁺ uptake, cytosolic charge balancing, enzyme activation, and maintenance of cell turgor (Szczerba et al. 2009; Loescher et al. 2011). As a result, the salt-tolerant plants can resume growing at a reduced level of Na⁺, in which plant hormones are believed to play important roles.

Plant hormones including auxin (AUX), gibberel-lin (GA), cytokinin (CK), abscisic acid (ABA), ethylene (ET), brassinosteroid (BR), jasmonate (JA), salicylic acid (SA), and strigolactone (SL), are small mobile chemical compounds and play important roles in plant growth, development, biotic and abiotic resistance (Bari and Jones 2009; Fujita et al. 2006; Horvath et al. 2007; Wang et al. 2016; Ma and Dong 2020). It is believed that the plant hormones JA, SA, ABA, BR, and ET play crucial roles in plant responses to biotic and abiotic stresses (Ku et al. 2018). For example, it was reported that salt stress induced ABA accumulation, and ABA treatment improved salt tolerance in plants (Chen et al. 2006; Park et al. 2008). Overexpression of the ABA biosynthesis related gene encoding 9-cis-epoxycarotenoid dioxygenase (NCED) or repression of the osmotic responsive gene (*LOS5*) led to an increase of ABA concentration and enhancement of salt tolerance (Lu et al. 2013; Zhang et al. 2009, 2016).

Ethylene is known to be involved in regulating salt tolerance in plants. However, the functions of ethylene in dicotyledonous plants and monocotyledonous plants are different (Morgan and Drew 1997; van Loon et al. 2006). In dicotyledonous plant *Arabidopsis*, ethylene treatment increased salt tolerance, and the *ethylene overproducer1* (*eto1*) mutant exhibited ethylene accumulation and promoted salt tolerance by reducing Na⁺ delivery from root to shoot (Jiang et al. 2013; Peng et al. 2014). The ethylene insensitive mutants *ethylene response1-1* (*etr1-1*), *ethylene insensitive4-1* (*ein4-1*), *etr2-1*, *ein2-1*, *ein2-5*, and *ein3-1* showed increased salt sensitivity, while the ethylene sensitive or over-sensitive mutants *etr1-7*, *constitutive triple response1* (*ctr1-1*), and *EIN3 binding F-box1-1* (*ebf1-1*) had increased salt tolerance (Achard et al. 2006; Cao et al. 2007, 2008; Lei et al. 2011; Wang et al. 2008). These results indicated that ethylene application, overproduction, or signaling can positively improve salt tolerance in the dicotyledonous plant. However, in monocotyledonous plant such as rice, lack of *MHZ6/OsEIN3-LIKE1* (*OsEIL1*) and *OsEIL2* caused ethylene insensitive and improved salt tolerance, but their overexpression lines showed ethylene over-sensitivity and decreased salt tolerance through the regulation of K⁺ transport and Na⁺ uptake in the roots (Yang et al. 2015). Unfortunately, less is known about the regulatory functions of ethylene response and signaling in salt sensitivity of wheat plants.

Considering the deterioration of continuous soil salinity on bread wheat for food supplement in the world, there is an urgent need to improve salt tolerance of the wheat plants to meet future global food requirements. In this study, the seeds of bread wheat cultivar Luyuan502 were mutagenized by ethylmethylsulfonylate (EMS) to generate salt-tolerant mutants. By screen of the mutant plants, we obtained the salt-tolerant lines with ethylene insensitive.*
Materials and methods

Plant material, growth condition, and EMS mutagenesis

The bread wheat cultivar Luyuan502 (approved by the Chinese National Crop Variety Examination and Approval Committee 2011) was used for EMS mutagenesis. Over 90,000 seeds of ‘Luyuan502’, designated as M₀ seeds, were selected and sterilized in 2% NaClO for 10 min, washed in sterile water for 3 min. After washing 5 times, the seeds were soaked in sterile water in darkness for 24 h. The surface sterilized seeds were incubated in 0.4% EMS solution with gentle agitation (50 rpm) for 24 h at room temperature. After washing 3 times in distilled water, the seeds were sowed in the saline soil of farm in Maotuo Village, Lijin County of Shandong Province, China. First generation of seeds (M₁) of every single plant with excellent growth was harvested in the saline soil field with 0.3–0.7% salts, separately. Similarly, the additional four generations (M₂–M₅) of seeds were obtained in the salinized field in the following years.

Ethylene sensitivity assays

Surface sterilized seeds in 2% NaClO were placed in the petri dish filled with two layers of sterile filter paper and supplemented with sterile ethylene precursor 1-amino-cyclopropane-1-carboxylate (ACC) at 300 μM. The petri dishes were wrapped in aluminum foil and incubated for 24 h or 6 d at room temperature. Ethylene insensitive mutants were picked up from the screen by measuring seedling growth and root length of the etiolated seedlings. Three replicates were taken, and at least 10 seeds were examined in each replicate.

Salt stress sensitivity assays and physiological examinations

Sterilized seeds were placed in the petri dish filled with two layers of filter paper and supplemented with 150 mM NaCl for salt stress assay. The petri dishes were incubated in the growth chamber at 25 °C under 16 h light/8 h dark for 24 h or 6 days. Salt-tolerant mutants were obtained from the screen by measuring the hypocotyl and root lengths of wheat seedlings. The experiment was repeated for three times, and at least 10 samples were examined in each replicate. The seedlings were then used for examination of the SOD, POD, and CAT activities and the productions of malonic dialdehyde (MDA) and proline (Pro) as previously described (He et al. 1997; Zhang et al. 2019; Zou 2000). Three replicates were used in the assays.

qRT-PCR analysis

Total RNA was extracted and the cDNA was synthesized as previously described (Ma et al. 2015). The TaACTIN (GenBank: AB181991) was used as the endogenous control. The qRT-PCR was performed according to a previous report (Ma et al. 2015). Three biological replicates were used in the experiments.

Transcriptome sequencing

The wild type wheat ‘Luyuan502’ and the salt-tolerant mutant lines 2–7 and 1–29 were used for transcriptome analysis. The 2-week-old wheat seedlings treated with 150 mM NaCl for 24 h or untreated were collected and used for sequencing by Genedenovo Biotechnology Co., Ltd (Guangzhou, China). The genes with fold change ≥ 2 and False Discovery Rate (FDR) < 0.05 were defined as significant Differentially Expressed Genes (DEGs). Their Gene Ontology (GO) functions and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were further detected according to the GO database (http://www.geneontology.org/) and KEGG database (http://www.genome.jp/kegg/), respectively. The transcription factors were identified using software iTAK1.2 (Zhang et al. 2016).

Characterization of the wheat ERFs in response to ethylene and salt stress

The ERFs in response to ethylene and salt stress were identified according to the results of transcriptome sequencing. The ERFs properties were analyzed using ProtParam (http://web.ExPASy.org/ProtParam/). Multiple sequence alignments of the ERFs were created using Clustal X, and then used as the input to construct phylogenetic tree (Tamura et al. 2011). GSDS (http://gsds.cbi.pku.edu.cn/) was used to analyze the exon—intron structure of the ERFs, and SMART (http://smart.embl-heidelberg.de/) was used to identify the GCC-box binding domain in the ERFs (Zou et al. 2018). Heatmap of the ERFs expression levels was constructed by the software omicshare (https://www.omicshare.com/tools/). The red color indicated the high-expressed genes, and blue represented the low-expressed genes.

Statistical analyses

Statistical analyses were performed by SAS, and the statistical significance of the difference was evaluated by ANOVA.
Results

Development of a new salt-tolerant wheat library

In order to induce salt-tolerant bread wheat lines, the bread wheat cultivar Luyuan502 was mutagenized by chemical mutagen EMS. Firstly, about 500 seeds of ‘Luyuan502’ were mutagenized by 0%, 0.2%, 0.4%, 0.6%, 0.8%, and 1.0% EMS, respectively. The EMS-mutagenized seeds were sowed, germinated, and grown in a saline-alkali soil (0.3–0.7% salinity). When EMS concentrations were used at 0%, 0.2%, 0.4% and 0.6%, the germination rates were about 71%, 69%, 58% and 55%, respectively (Supplementary Material S1). The surviving rate was nearly 0% when the EMS concentration was more than 0.8% (Supplementary Material S1). Although the germination rate at 0.6% of EMS was not obviously lower than that of 0.4% EMS treatment, the germination uniformity was significantly decreased at 0.6% EMS treatment. In order to obtain more mutated seedlings, 0.4% EMS was chosen as the optimal concentration for mutagenesis. In total, over 90,000 seeds of ‘Luyuan502’ were treated and sowed in the salinity field, and about 60,000 M1 plants were obtained. Among them, about 2000 M1 plants showing strong salt tolerance in the saline soil (Fig. 1a–d) were harvested. A single-seed descent population was developed for further screening.

To select high yield, the mutant lines and the wild type ‘Luyuan502’ were planted in the salinity field with same quantity (150 g) and same area (20 m²) in the Maotuo experimental field (Fig. 1e–h). Mainly based on crop yield, 5 salt-tolerant lines including 1–98, 2–7, 1–105, 1–119, and 1–29 from M3 generation were selected for further study, and the data were summarized in the Supplementary Material S2. The results indicated that the production of the salt-tolerant lines 1–29 and 1–105 was increased by 20%—30%, and the lines 2–7 and 1–119 increased about 10–20%. Although the production of M4 and M5 generations showed some variations among these lines, most of the lines kept increased crop yield in the saline soil (Supplementary Material S2).

To further examine the salt tolerance of the wheat plants, the M3 seeds were soaked and cultivated in the petri dish with 0, 50, 100, 200, 300 mM NaCl for 48 h, and the root lengths of the seedlings were measured and compared. The results indicated that the root length was getting shorter with the increase of NaCl concentration, and 150 mM NaCl was selected as the optimal concentration for the salt tolerance examination. Among 2,000 lines of the wheat mutants, 430 lines including 1–98, 2–7, 1–105, 1–119 and 1–29 exhibited longer roots than wild type after salt treatment (Fig. 2a, b).

Ethylene sensitivity detection and salt resistance confirmation of wheat mutants

As ethylene insensitivity is highly related with stress tolerance in plants (Wang et al. 2008; Achard et al. 2006; Cao et al. 2007, 2008; Lei et al. 2011), the ethylene sensitivity of the wheat lines was examined. The wheat seeds were soaked and cultivated in the petri dish supplemented with the ethylene precursor ACC at different concentrations (0, 100, 200, 300, and 400 μM) for 48 h, and the longest seedling root lengths were measured. As expected, the root lengths were decreased as ACC concentration increased, and 30 out of 430 salt-tolerant lines including the lines of 1–98, 2–7, 1–105, 1–119 and 1–29 exhibited ethylene insensitivity at seedling stage (Fig. 2c, d).

The salt stress responses of the soil-grown plants of the selected wheat lines were examined. One-week-old seedlings were grown in pots and watered with different concentrations of NaCl (0, 100, 200, 300 mM) for 30 days. The fresh weight and dry weight were measured, and the fresh weight and dry weight of the salt-tolerant lines were higher than those of the wild type (Fig. 3a–c).

The MDA and Pro contents, and the SOD, CAT and POD activities were also compared between the salt-tolerant lines and the wild type. The results showed that salt treatment increased the MDA, Pro contents and the SOD, CAT, POD activities in all the samples (Fig. 3d–h). The Pro contents and the SOD, CAT and POD activities in the salt-tolerant lines were significantly higher than those of the wild type, and the MDA contents of the salt tolerant lines were significantly lower than that of the wild type, while most of them had no difference when the NaCl treatment was absent (Fig. 3d–h). All these results indicated that the wheat lines enhanced their salt tolerance by accumulation of Pro content, increase of SOD, CAT and POD activities, and inhibition of MDA production when challenged by salt stress.

Transcriptomic analysis of the responsive genes in the wheat mutant lines

In order to explore the salt-tolerant mechanism in the selected mutants, the lines 2–7 and 1–29 were chosen for transcriptome analysis, and ‘Luyuan502’ was used as control. The 2-week-old seedlings of the lines treated with 150 mM NaCl and untreated samples were collected and used for RNA-seq analysis. Based on the comparisons between the untreated and the salt treated samples from the same line, the transcripts with fold change ≥ 2 and FDR < 0.05 were defined as significant DEGs. A venn diagram of DEGs in multiple comparisons of WT-vs-WT-S (salt treated WT samples), 2–7-vs-2–7-S (salt treated 2–7 samples), and 1–29-vs-1–29-S (salt treated 1–29 samples) showed 3278 DEGs overlapped (Fig. 4a). The GO and KEGG enrichment analysis
indicated that the overlapped DEGs were mostly affected and mainly involved in photosynthesis, biosynthesis of secondary metabolites, cyanoamino acid metabolism, carotenoid biosynthesis, thiamine metabolism, and cutin, suberine and wax biosynthesis pathways including the genes encoding CHLOROPHYLL a-b BINDING PROTEINS (CABs), PEROXIDASEs (PERs/PODs), BETA-GLUCOSIDASEs (BGLUs), CYTOCHROME P450 707s (CYP707s), ZEAANTHIN EPOXIDASEs (ZEPs), and HYDROXYMETHYLPYRIMIDINECs (THICs) (Fig. 4b, c; Supplementary Materials S3–S5).

qRT-PCR verification of the transcriptome results

To confirm the transcriptome sequencing results, 12 DEGs selected based on the multiple comparisons of WT-vs-WT-S, 2–7-vs-2–7-S, and 1–29-vs-1–29-S were examined for their relative expression levels by qRT-PCR (Fig. 5; Supplementary Materials S3–S5). The sequences of the selected DEGs were listed in the Supplementary Material S6, and the used primes were showed in the Supplementary Material S7. The relative expression levels of the selected genes in the wheat lines with different
NaCl treatments were examined, and the results showed high consistence with that of the transcriptome analysis (Fig. 4).

In addition, some stress-related genes including *TaNHX1*, *TaNHX3*, *TaNHX7*, *TaHAK1*, *TaZIP29*, *TaGSVI*, *TaSALT-1*, *TaSALT-2*, and *TaSALT-3* (Kawaura et al. 2008; Lu et al. 2014), were also examined. The results indicated that the expressions of the salt-related genes in the selected mutant lines were higher than those of the wild type (Fig. 6f–o).

As the salt-tolerant wheat lines exhibited decreased ethylene sensitivity, the relative expression levels of the ethylene-related *ETHYLENE RESPONSE FACTORS* (ERFs) (*TaERF1*, 2, 3, 4 and 6) under different salt treatments were examined (Fig. 6a–e). The results indicated that most of the mutant lines had higher expression levels of the *TaERFs* even without NaCl treatment, and the salt stress significantly increased the expression levels of the *ERFs* in most of the salt-tolerant mutant lines (Fig. 6a–e).

Expression characteristics and structural variations of novel ERF genes in response to ethylene and salt stress in wheat

In addition to the known wheat ERFs (*TaERF1, 2, 3, 4* and 6), analysis of the transcriptome data identified 9 novel wheat *ERFs* in response to ethylene and salt stress in this study. The CDS sequences and full DNA sequences of the wheat *ERFs* were showed in the Supplementary material 8. The basic biochemical properties of the ERFs including molecular weight, theoretical pI, instability index, and aliphatic index were detected using ProtParam (http://web.
ExPASy.org/ProtParam/), and the results were summarized in the Supplementary material 9. The phylogenetic map and exon–intron structure analysis showed that the ERFs have diverse gene structures (Fig. 7a). However, these 9 ERFs all contained conserved GCC-box binding domain, indicating that these ERFs may be controlled by the same trans-acting factor (Fig. 7b). Heatmap analysis showed that the expression levels of the ERFs TRIAE_CS42_5DL_TGACv1_432926_AA1394650 and TRIAE_CS42_6AS_TGACv1_486327_AA1559760 were decreased significantly after salt treatment, while the expression levels of the other 7 ERFs increased after salt treatment, suggesting that they may play different roles in ethylene regulated salt tolerance in wheat (Fig. 7c).

**Discussion**

**Evaluation of the genetic screen for the salt-tolerant wheat lines with ethylene insensitivity**

As wheat is moderately salt tolerant (Munns and Tester 2008), it is of great importance to generate salt-tolerant varieties to improve the wheat production. In this study, new wheat germplasm was generated by EMS mutagenesis from a popular wheat variety Luyuan 502 in China. The wheat variety is a semi-winter, mid-maturing variety with cuboid spikes, long awns, white shells, white grains, strong growth, and excellent resistance to cold stress, rust and powdery mildew diseases, and wildly planted in the northern part of Chinese Huanghuai winter wheat region, including Shandong province, and the central and southern parts of Hebei and...
Shanxi provinces of China. In this study, a large mutant pool with over 60,000 M1 plants was generated in saline soil and screened for salt-resistant lines. Among them, 5 lines exhibited 10–30% increase of crop yield in M3 generation were selected, and their increased productions were also mostly detected in their M4 and M5 generations. The salt-resistant lines may contribute to the wheat production potentially in the future.

Ethylene is one of the plant hormones and plays an important role in plant salt tolerance (Yang et al. 2015; Bahieldin

Fig. 5 Expression profiles of the 12 salt-tolerant related DEGs in the wheat lines. a–l qRT-PCR assays for the relative expression of the 12 salt-tolerant related DEGs in different wheat plants treated with 150 mM NaCl for different periods (0–24 h). The statistical significance of the difference was confirmed by ANOVA at α = 0.05 level
et al. 2016; Cao et al. 2007). In dicotyledonous plants, the etiolated seedlings treated with the ethylene precursor ACC showed inhibition of root and hypocotyl elongation, swelling of the hypocotyl and exaggeration of the apical hook, which is known as ‘double response’ (Bleecker et al. 1988; Guzman and Ecker 1990). However, in monocotyledonous plants, the ethylene responses are more complex. For example, in rice, ethylene not only inhibits root growth, but promotes coleoptile growth of etiolated rice seedlings, which is collectively known as ‘double response’ (Ma et al. 2010, 2013, 2014; Yang et al. 2015). Unfortunately, less has been known about ethylene related responses in wheat. In the present study, it was found that ethylene insensitive mutants exhibit enhanced salt tolerance in the wheat (Fig. 2g–i). Based on the relationship between ethylene insensitivity and salt tolerance of the wheat seedlings, we screened the EMS-mutagenized pool and obtained the salt-tolerant wheat lines with alterations of the salt-related genes in different wheat plants treated with 150 mM NaCl for different periods (0–24 h). The statistical significance of the difference was confirmed by ANOVA at α=0.05 level.

**Transcriptomic analysis for alteration of the salt responsive gene expressions in wheat**

It was reported that salt stress induces ion imbalance and hyperosmotic effects, leading to increase of ROS concentration, damage of chloroplast, exaggeration of enzyme inefficiency, decrease of photosynthesis, and acceleration of photorespiration (Wang et al. 2015; Liu et al. 2019; Mallik et al. 2011). Plants also evolve in several responses to alleviate the negative effects of salt stress, which contribute to survival of plants in the salt stress. In this study, we investigated the responsive gene expressions of the salt-tolerant lines of the wheat by transcriptome analysis.

The transcriptomes of 2-week-old seedlings of the salt-tolerant wheat lines 2–7 and 1–29 treated with 150 mM NaCl were compared with those of the wild type samples. The multiple comparisons of WT-vs-WT-S, 2–7-vs-2–7-S, and 1–29-vs-1–29-S showed that the expressions of the genes encoding CABs, PERs/PODs, BGLUs, CYP707s, ZEPs, and THICs in photosynthesis, biosynthesis of secondary metabolites, cyanomino acid metabolism, carotenoid biosynthesis, thiamine metabolism, and cutin, suberine and wax biosynthesis pathways, were significantly changed (Fig. 4; Supplementary Materials S3–S5). These results provide clues suggesting that the alterations may be closely related with the salt tolerance of the wheat plants.

CABs bind chlorophyll a and b to make up light harvesting antenna complex, which absorbs light and transfer excitation energy to photosystems I and II in chloroplasts (Jansson 1999). CABs are encoded by multiple genes in higher plants. For instance, Arabidopsis contains 10 CAB encoding genes, and tomato has 16 CABs in the genome (Jansson 1999; Schwartz et al. 1991). Recently, it was reported that the expressions of the CAB encoding genes were induced by salt, indicating a significant role of CABs in salt resistance (Silva et al. 2016). In this research, 40 CAB encoding genes including TRIAE_CS42_1AL_TGACv1_000383_AA0010660, TRIAE_CS42_1AL_TGACv1_000708_AA0017440, TRIAE_CS42_1BL_TGACv1_030603_AA0095440, TRIAE_CS42_1BL_TGACv1_032695_AA0133030, TRIAE_CS42_1DL_TGACv1_061419_AA01949670, and TRIAE_CS42_1DL_TGACv1_061593_AA0199400 were involved in the salt-tolerant responses of wheat (Supplementary Materials S3–S5). Due to the limited information about the CAB encoding genes in salt response, more research definitely needs to be done in the future.

PERs/PODs are the enzymes that catalyze substrate oxidation with hydrogen peroxide as an electron receiver. In this research, 32 PER/POD encoding genes including TRIAE_CS42_5DL_TGACv1_434349_AA1434390, TRIAE_CS42_5DL_TGACv1_435881_AA1455860, TRIAE_CS42_5DL_TGACv1_436164_AA1458860, TRIAE_CS42_6AL_TGACv1_472947_AA1527400, and TRIAE_CS42_7AL_TGACv1_556904_AA1773120, were detected in the salt-tolerant response of the wheat plants (Supplementary Materials S3–S5).

The BGLUs belong to the subfamily I of glycoside hydrolases, which hydrolyze beta-glycosidic bonds to release terminal glucosyl residues from glycosides, oligosaccharides, and disaccharides (Ketudat Cairns and Esen 2010). The BGLUs were reported to release glucose from oligosaccharides in the cell wall, and consequently lead to change of the cell wall structures (Dharmawardhana et al. 1995). The BGLUs also activate defense compounds from inactive glycosides to defense against herbivores and fungi (Halkier and Gershenzon 2006). The BGLUs were also reported to release plant hormones from its inactive glyconjugates or release scent compounds from involatile precursor (Baba et al. 2017; Halkier and Gershenzon 2006). It was suggested that the specific function of a certain BGLU enzyme in plant growth, development, biotic and abiotic resistance depends on its expression pattern, substrate specificity, and different localization (Rouyi et al. 2014). The BGLU encoding genes can be induced by salt treatment. For example, the CsBGLU12 in Crocus sativus was significantly induced by salt, and its transient overexpression in tobacco leaves...
accumulated antioxidant flavonoids, which confer tolerance to salt stresses by alleviation of ROS accumulation (Baba et al. 2017). In this research, 41 BGLU encoding genes including TRIAE_CS42_2BL_TGACv1_130794_AA0418050, TRIAE_CS42_3B_TGACv1_222633_AA0768330, TRIAE_CS42_3B_TGACv1_223344_AA0780610, TRIAE_CS42_3DL_TGACv1_250039_AA0861010, TRIAE_CS42_4AS_TGACv1_307764_AA1023360, and TRIAE_CS42_5AL_TGACv1_374195_AA1193240, were detected in the wheat (Supplementary Materials S3–S5), suggesting that they may play important roles in salt response (Yousfi et al. 2016). In this study, 12 WRKY coding genes including TRIAE_CS42_7DS_TGACv1_623759_AA2055930, XLOC_076596, TRIAE_CS42_1AL_TGACv1_001348_AA0029060, TRIAE_CS42_1AL_TGACv1_002809_AA0045280, and TRIAE_CS42_1DL_TGACv1_062218_AA0210650, were detected (Supplementary Materials S3–S5), suggesting that they may play important roles in salt tolerance in wheat plants.

**The wheat ERFs in response to ethylene and salt stress**

ERF transcription factors regulate diverse biological processes in plant growth, development, abiotic and biotic stress responses by activating the genes with GCC-box in the promoter (Xu et al. 2011; Gutterson and Reuber 2004). To date, 117 ERFs have been identified in bread wheat and only a few ERFs including TaERF1, TaERF3, TaPIE1, TaPIEP1, TaERF4, and TaERF8-2B have been characterized. The TaERF8-2B was reported to play roles in plant growth and development, and regulation of plant architecture and yield related traits, closely associated with plant height, heading date and 1000 kernel weight (TKW) (Zhang et al. 2020). Overexpression of TaPIE1 in wheat accumulated high soluble sugars and proline contents, and improved plant tolerance to freezing and the necrotrophic pathogen *Rhizoctonia cerealis* (Zhu et al. 2014). Overexpression of the transcription factor TaPIEP1 in wheat enhanced resistance to the fungal pathogen *Bipolaris sorokiniana* (Dong et al. 2010).

In addition, the wheat ERF transcription factors play roles in abiotic stress. Overexpression of TaERF3 in bread wheat promoted tolerance to salt and drought stresses (Rong et al. 2014). TaERF1 was reported to contribute to drought, cold, and salt tolerance in transgenic *Arabidopsis* plants (Xu et al. 2007). In contrast, it was suggested that TaERF4 might have an opposite role in plant abiotic stress tolerance. Overexpression of TaERF4 in *Arabidopsis* enhanced salt sensitivity (Dong et al. 2012). These studies suggested diverse functions of the ERF transcription factors in plant stress responses (Dong et al. 2012).

In addition to the known wheat ERFs (TaERF1, 2, 3, 4 and 6), this study reported the identification of 9 novel wheat ERFs in response to ethylene and salt stress based on transcriptome sequencing. Heatmap analysis showed that the expression levels of 2 ERFs (TRIAE_CS42_5DL_TGACv1_432926_AA1394650 and TRIAE_CS42_6AS_TGACv1_486327_AA1559760) were decreased significantly after salt treatment, suggesting they may play negative role in ethylene regulated salt tolerance in wheat (Fig. 7c). At the same time, the expression levels of the other 7 ERFs including TRIAE_CS42_3DL_TGACv1_250510_AA0869450, TRIAE_CS42_6DL_TGACv1_526341_AA1680010, TRIAE_CS42_7AL_TGACv1_556681_AA1768690, TRIAE_CS42_7AS_TGACv1_569387_AA1814810, TRIAE_CS42_7DL_TGACv1_606411_AA2010000, TRIAE_CS42_7DS_TGACv1_621751_AA2025220, and TRIAE_CS42_7DS_TGACv1_623962_AA2057840, were increased significantly after salt treatment, indicating their positive response in ethylene regulated salt tolerance in wheat (Fig. 7c). Comparing with the previous studies, it was suggested that the wheat ERFs may function differently in regulation of stress responses. As most of ERF transcription factors may function in abiotic tolerance (Xu et al. 2011), it is possible to improve the salt tolerance...
of the wheat plants by manipulating the ERFs levels in response to ethylene and salt stress in the future.

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Author contributions QM worked on transcriptome analysis, statistical analysis, and writing of first version of the manuscript. HZ contributed on EMS mutagenesis and physiological experiments. XS and CS worked on qRT-PCR analysis. YW worked on mutant screening. CHD participated in design of the experiments and revision of the manuscript. All authors have read and approved the manuscript.

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Data availability The FASTQ files of raw data were uploaded to the NCBI Sequence Read Archive (SRA), and the SRA study accession is PRJNA549107.

Compliance with ethical standards Conflict of interest We declare that the authors of this paper have no conflict of interest.

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