Comparative proteomic analysis for revealing the advantage mechanisms of salt-tolerant tomato (*Solanum lycoperscium*)

Qiang Wang [1, 2] , Baike Wang [2] , Huifang Liu [2] , Hongwei Han [2] , Hongmei Zhuang [2] , Juan Wang [2] , Tao Yang [2] , Hao Wang [Corresp., 2] , Yong Qin [Corresp. 1]

[1] College of Horticulture,Xinjiang Agricultural University, Urumqi, China

[2] Institute of Horticulture Crops, Xinjiang Academy of Agricultural Sciences, Urumqi, China

Corresponding Authors: Hao Wang, Yong Qin
Email address: wanghao183@163.com, xjndqinyong@126.com

Salt stress causes the quality change and significant yield loss of tomato. However, the resources of salt-resistant tomato were still deficient and the mechanisms of tomato resistance to salt stress were still unclear. In this study, the proteomic profiles of two salt-tolerant and salt-sensitive tomato cultivars were investigated to decipher the salt-resistance mechanism of tomato and provide novel resources for tomato breeding. We found high abundance proteins related to nitrate and amino acids metabolismsin the salt-tolerant cultivars. The significant increase in abundanceof proteins involved in Brassinolides and GABA biosynthesis were verified in salt-tolerant cultivars, strengthening the salt resistance of tomato. Meanwhile, salt-tolerant cultivars with higher abundance and activity of antioxidant-related proteins have more advantages in dealing with reactive oxygen species caused by salt stress. Moreover, the salt-tolerant cultivars had higher photosynthetic activity based on overexpression of proteins functioned in chloroplast, guaranteeing the sufficient nutrient for plant growth under salt stress. Furthermore, three key proteins were identified as important salt-resistant resources for breeding salt-tolerant cultivars, including sterol side chain reductase, gamma aminobutyrate transaminase and starch synthase. Our results provided series valuable strategies for salt-tolerant cultivars which can be used in future.

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2 **Salt-tolerant tomato (*Solanum lycopersicum*)**

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4 Tao Yang [2], Hao Wang [2*], Yong Qin [1*]

6 [1]College of Horticulture,Xinjiang Agricultural University, Urumqi 830052,China ;

7 [2]Institute of Horticulture Crops, Xinjiang Academy of Agricultural Sciences, Urumqi 830091, China;

9 *Corresponding author: Hao Wang , E-mail: wanghao183@163. com

10 *Corresponding author:Yong Qin , E-mail: xjndqinyong@126.com

ABSTRACT

29   Salt stress causes the quality change and significant yield loss of tomato. However, the resources

30   of salt-resistant tomato were still deficient and the mechanisms of tomato resistance to salt stress

31   were still unclear. In this study, the proteomic profiles of two salt-tolerant and salt-sensitive

32   tomato cultivars were investigated to decipher the salt-resistance mechanism of tomato and

33   provide novel resources for tomato breeding. We found high abundance proteins related to

34   nitrate and amino acids metabolisms in the salt-tolerant cultivars. The significant increase in

35   abundance of proteins involved in Brassinolides and GABA biosynthesis were verified in salt-

36   tolerant cultivars, strengthening the salt resistance of tomato. Meanwhile, salt-tolerant cultivars

37   with higher abundance and activity of antioxidant-related proteins have more advantages in

38   dealing with reactive oxygen species caused by salt stress. Moreover, the salt-tolerant cultivars

39   had higher photosynthetic activity based on overexpression of proteins functioned in chloroplast,

40   guaranteeing the sufficient nutrient for plant growth under salt stress. Furthermore, three key

41   proteins were identified as important salt-resistant resources for breeding salt-tolerant cultivars,

42   including sterol side chain reductase, gamma aminobutyrate transaminase and starch synthase.

43   Our results provided series valuable strategies for salt-tolerant cultivars which can be used in

44   future.

46   **Keywords:** Tomato (*Solanum lycopersicum*); Salt-tolerant; Proteome; Salt tolerant variety

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INTRODUCTION

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57 Soil salinization is a worldwide problem affecting agricultural production. With the destruction

58 of soil caused by the expansion of the global industrial scale, nearly 20% of cultivated land and

59 nearly half of the irrigated land are threatened by salt damage to varying degrees in the world.

60 Soil salinization has become a major environmental problem which has been widely realized

61 (Rozema and Flowers 2008). The increasing trend of salinization poses a great threat to the

62 sustainable development of agriculture. Currently, the agricultural improvement measures of

63 salinization soil mainly include rational fertilization, increasing organic fertilizer and restraining

64 soil salt super accumulation(Chen, 2011).However, the cultivation of saline-tolerant crop

65 varieties is still an important method, which is meaningful to the improvement and utilization of

66 salinized soil. As an important cash crop in the world, tomato growing was limited by various

67 factors relevant to salt stress, resulting in a loss of yield. However, the salt-tolerance mechanism

68 of tomato is still unclear and need further in-depth researches.

69 The major harmful ions in saline and alkaline soils is Na^+. The accumulation of Na^+ in soils

70 could result in osmotic stress (Xiong & Zhu, 2002; Munns& Tester, 2008), ion toxicity (Tester &

71 Davenport, 2003) and oxidative stress (Hasegawa et al., 2000; Xiong & Zhu, 2002), which

72 profoundly influence the growth and development of plants. The excessive accumulation of Na^+

73 in plant cells results in the destruction of the dynamic balance between the generation and

74 clearance of oxygen free radicals, and leads to or intensifies the membrane lipid peroxidation and

75 membrane lipid defatination (Senaratna et al.,1985), which is harmful to the normal

76 physiological progresses of plants. Additionally, excessive Na^+ decreases the concentration of

77 Ca^{2+} in the plasma membrane, resulting in membrane structure destruction and changes in

78 function, and intracellular exosmosis of potassium, phosphorus and organic solute (Tuna et al.,

79 2007), which inhibited plant growth and development. It has been proved that salt stress can

80 influence the utilization rate of starch, protein, fatty acids and other macromolecular substances

81   in seeds, resulting in seed germination failure or delayed germination (Lovato et al.,1994;

82   Ungar,1996). Meanwhile, the repair and reconstruction of cell membrane system of seeds were

83   also impaired under saline-alkali stress. The membrane ion selective absorption capacity is

84   decreased under salt stress, accompanying with ion toxicity and generation of free radicals which

85   further damage the seed colloidal structure and slow down the development of seeds

86   (Almansouri et al.,2001; Chartzoulakis and Loupanaki 1997). Furthermore, salt stress could

87   significantly influence the structure of plasma membrane. Salt stress can also lead the formation

88   of large vacuoles, local expansion of endoplasmic reticulum, decrease in chloroplast numbers

89   and enlargement of mitochondria (Mitsuya et al., 2000; Bruns and Hecht-Buchholz 1990).

90   Currently, various mechanisms of plant tolerance to salt stress have been elucidated, including

91   reprogramming the photosynthetic pathway (Allakhverdiev et al.,2002; Mittal et al., 2012;Wang

92   et al.,2021), enhancing the antioxidant protection ability (Gupta et al.,1993;Parvin et

93   al.,2019;Faizan et al.,2021) and synthesis and accumulation of osmotic regulating substances

94   (Yang et al., 2007;Muhammad et al., 2021). It has been reported that salt stress induce the

95   increase in activity of SOD and APX, which plays important roles in the antioxidant protection

96   system of plants (Zhang and Mu 1993). Compared with wild-type tobacco, salt-resistant cultivars

97   have higher photosynthetic efficiency and are more resistant to salt stress (Gupta et al., 1993).

98   Under salt stress, plants usually need to synthesize and accumulate a large amount of solutes

99   with active and non-toxic osmotic effects to reduce water potential (Chen, 2011), allowing cells

100   to absorb water in a hypotonic environment. Moreover, plants also activate multiple signaling

101   pathways to resist the harms caused by salt stress, including salt oversensitive (SOS) signal

102   transduction pathway, jasmonic acid, mitogen-activated protein kinase (MAPK), and abscisic

103   acid (ABA) signaling pathways (Xiong et al., 2002; Zhu, 2002; Colcombet& Hirt, 2008).

104   Therefore, plants regulate salt tolerance through a complex network of resistance response.

105   Tomato is a worldwide vegetable crop whose tolerance mechanism and proteomics of salt stress

106   have been investigated extensively and deeply (Manaa, A., et al.,2013; Gong et al.,2014).

107 Comparative proteomic analyses are highly effective in the identification of protein changes that

108 are associated with genotypic properties. For instance, an iTRAQ analysis of proteomes from

109 Maize inbred lines at salt-tolerant genotype F63 and the salt-sensitive genotype F35 showed that

110 a large number of proteins was affected by salt in the two accessions (Cui et al.,2015). Those

111 differentially expressed proteins may be helpful for further elucidating salt tolerance mechanisms.

112 The salt-tolerant introgression line IL7-5-5 was constructed by using 76 wild tomato Solanum

113 Pennellii LA716 as donor and cultivated tomato M82 as acceptor. The salt-tolerant line IL7-5-5

114 was screened by repeated salt stress method at seedling stage. breeders observed that IL7-5-5

115 plants were salt-tolerant while M82 plants were susceptible to salt stress. In this study, we found

116 that various proteins relevant to salt resistance were highly expressed in salt-tolerant cultivars,

117 resulting in series advantages of ST in permeability regulation, maintaining normal

118 photosynthesis and removing reactive oxygen species produced by salt stress. Our research

119 provides a new blueprint for breeding salt tolerance tomato.

120 **MATERIALS AND METHODS**

121 **Plant material and Experimental design**

122 The experiment was carried out in the sunlight greenhouse of Xinjiang Academy of Agricultural

123 Sciences in April 2019. The seeds of salt-tolerant infiltration system IL7-5-5 (ST) and salt-

124 sensitive M82 (SS) were seeded in peat, perlite and vermiculite (volume ratio 1:1:1) substrates,

125 seedling raising in conventional greenhouse. The roots were cleaned when the seedlings grow to

126 the four-true-leaf stage, then transplanted the plant into 1/2 concentration Hoagland standard

127 nutrient solution for hydroponic culture. The seedlings were cultivated at 25/18°C (day/night)

128 with a relative humidity of 40%, and at a photoperiod of 14h/10 h (day/night). The oxygen was

129 supplied to culture chambers three times everyday and 30 minutes every time. The evaporated

130 water was replenished to its initial volume every two days, this culture method last for one week

131 and the treatment started after this.The following experimental groups were arranged with 3

132 replicates and treated as follows:(1)CK: 1/2 Hoagland Nutrient Solution; (2) Salt Stress

133 Treatment:1/2 Hoagland nutrient solution with 200 mmol·L^{-1} NaCl. Sample the location of the

134 same part of the material to be tested at 0, 1, and 12 h after treatment. The leaves were selected

135 and stored in the refrigerator at -80 ℃. All sample collection and preparation procedures were

136 performed in triplicate, and each independent biological experiment has 3 technological

137 replicates. Data were exhibited as the means±standard deviations (SDs).

138 **Determination of Physiological indicators**

139 Tomato leaves at 0 h and 1 h after salt treatment were used for Superoxide dismutase (SOD)

140 activity, Polyamine oxidase (PAO) activity, Peroxidase (POD) activity, Polyphenol oxidase

141 (PPO) activity, Soluble sugar content and H_2O_2 content assays by physiological trait kits (Suzhou

142 Comin Biotechnology Co. Suzhou, China) according to the manufacturer's instructions. A

143 completely randomized block design was used, and all experiments had three replicates.

144 Experimental data were analyzed by two-way analysis of variance (ANOVA) and Tukey ($P <$

145 0.05) comparisons were used to analyze differences.

146 **Protein extraction,Trypsin digestion and Isobaric Labeling**

147 Frozen tissue from tomato leaves was ground in liquid nitrogen using a mortar and pestle. Dry

148 samples were mixed with lysis buffer (1% TritonX-100, 10 mM dithiothreitol, 1% protease

149 inhibitor cocktail and 2 mM EDTA) and then processed three times on ice using a high intensity

150 ultrasonic processor. After centrifugation (12,000 g, 4°C, 10 min) using Tris-saturated phenol

151 (pH 8.0) and transferring the supernatant, add at least 4 volumes of ammonium sulfate saturated

152 methanol and allow the protein to precipitate overnight at 20°C. Remove the supernatant by

153 centrifugation (4°C, 10 min) and wash three times with ice-cold acetone. Protein concentration

154 was determined using the BCA kit. Protein was precipitated at 20% (m/v) TCA. Protein

155 precipitates recovered after vortex mixing incubation (4°C, 2h) and centrifugation (4500g, 4°C,

156 5min) were washed with pre-chilled acetone, and the dried protein samples were re-dissolved in

157 200nM TEAB and digested overnight with trypsin (1:50 w/w). Samples were reduced with 5

158 mM dithiothreitol (37°C, 60 min) and alkylated with 11 mM iodoacetamide (room temperature,

159 45 min) in the dark.   Peptide Isobaric labeling was performed using a TMT kit (Thermo Fisher

160 Scientific, Waltham, MA, USA), with SS samples labeled with TMT tags 126, 127 and 128, and

161 ST samples using TMT tags 129, 130 and 131.

Protein Identification by LC-MS/MS

163 Separation of samples into fractions by high pH reversed-phase HPLC using Agilent 300 Extend

164 C18 column (5 μm particles, 4.6 mm I.D., 250 mm length). The tryptic peptides were dissolved

165 in solvent A (0.1% formic acid, 2% acetonitrile in water), then loading all the solution into a

166 home-made reversed-phase analytical column. The whole operation was conducted on an EASY-

167 nLC 1000 Ultra Performance Liquid Chromatography system with a constant flow rate of 400

168 nl/min, and the detail information was followed: the gradient was comprised of an increase from

169 6% to 23% solvent in 98% acetonitrile over 26 min, 23% to 35% with 8 min and then climbing

170 to 80% in 3 min, finally, hold at 80% for 3 min. The information of instrument applied during

171 experiment was followed: electrospray voltage of 2.0 kv, m/z scan range of 350 to 1800 for full

172 scan, and intact peptides detecting resolution of 70,000. NCE was used to select peptides and the

173 Orbitrap with a resolution of 17,500 was used to detect the fragments.

Data analysis

175 Maxquant search engine (vs 1.6.3.3, Cox and Mann, 2008) and uniprot database was use to

176 process MS/MS data. Several protein types should be classified for accurate analysis and the

177 detail information was followed: carbamidomethyl on Cys was recongnized as fixed

178 modification and acetylation on protein N-terminal or oxidation on Met and deamidation (NQ)

179 were classified as variable modifications. TMT-6plex quantification has also been performed.

180 More software operation parameters were followed: mass tolerance for fragment ions was 0.02

181 Da, first search of mass tolerance for precursor ions was 20 ppm, main search of mass tolerance

182 for precursor ions was 5 ppm, FDR was adjusted to $< 1\%$ and minimum score for modified

183 peptides was set >40.

Enrichment Analysis

185 Gene ontology and KEGG pathway enrichment analyses were carried out by R packages cluster

186 profiler. Proteins were classified by GO annotation into three categories: biological process,

187 cellular compartment and molecular function. For each GO category, a two-tailed Fisher's exact

188 test was employed to test the enrichment of the differentially expressed protein against all

189 identified proteins. Both pathway and GO terms with a p-value < 0.05 is considered significant.

190 Furthermore, wolfpsort were used for a subcellular localization predication to predict the protein

191 subcellular localization.

192 **Protein-protein Interaction Network**

193 All candidate proteins were searched against the STRING database version 10.1 for protein-

194 protein interactions. Only interactions between the proteins belonging to the searched data set

195 were selected, thereby excluding external candidates. Interaction network form STRING was

196 visualized in Cytoscape 3.0. The settings used in STRING database was shown as below:

197 Network type: full STRING network (the edges indicate both functional and physical protein

198 associations); meaning of network edges: evidence; active interaction sources: Textmining、

199 Experiments、Databases、Co‑expression、Neighborhood、Gene Fusion and Co‑occurrence.

200 **qRT-PCR verification of gene expression of autophagy-related proteins**

201 The expression of important salt related proteins (A0A0C6G3Q8, A0A3Q71RF5 and

202 A0A37J2V8) was investigated by real-time quantitative polymerase chain reactions (RT-qPCR).

203 Primer 6(v6.24) Designer was used to design the RT-qPCR specific primers (**Supplementary**

204 **Table1**). The method of total RNA extraction, quantification and cDNA synthesis were

205 described previously. Vieira et al(2016). All experiments have been carried out at least three

206 times with three independent biological replicates. The relative expression levels of target genes

207 was calculated by the method of fold change of $2^{-\triangle\triangle Ct}$ value (Schmittgen et al.,2008).

208 **RESULTS**

209 **Observable physiological changes between Salt tolerant (ST) and Salt sensitive (SS)**
210 **cultivars**

211 One hour after imposing, the content of carbohydrate, H_2O_2 and total protein in both Salt-

212 sensitive and Salt-tolerant cultivars were determined. The results showed that no significant

213 change was observed in the content of carbohydrate among both cultivars (Fig.1A). Moreover,

214 there was no significant difference in the content of H_2O_2 between in Salt-tolerant tomato

215   compared with the Salt-sensitive tomato (Fig.1B).One hour after imposing, the content of H_2O_2

216   in Salt-sensitive tomato was still significantly higher than that in Salt-tolerant cultivars (Fig.1B),

217   which may cause damage to these cultivars. In parallel, there was no significant difference in

218   content of total protein among both Salt-sensitive and Salt-tolerant cultivars. However, 1h after

219   imposing, the content of total protein in Salt-tolerant cultivars was significantly increased to a

220   higher level than Salt-sensitive cultivars (Fig.1C).

221   **Global proteome analysis of Salt tolerant cultivars (ST) and Salt sensitive (SS)   cultivars**

222   To further elucidate the mechanism of resistance to salt stress, mass spectroscopy based

223   proteomics was performed. Both Salt tolerant cultivar (ST) and Salt sensitive (SS)   cultivar

224   treated after 0h (ST and SS), 1h (ST 1h and SS 1h) and 12h (ST 12h and SS 12h) were harvested

225   for proteomic analysis, and each treatment had three replicates. Furthermore, 7663 function

226   proteins were identified among all samples and analyzed by variation analysis(**Supplementary**

227   **Table 2**).

228   To investigate the influences of salt stress on cultivars (SS and ST), two algorithms were further

229   performed, including principle components analysis (PCA) and unsupervised cluster analysis

230   (Fig.2A, 2B). First, PCA analysis showed an observable separation between all six treatments

231   demonstrating that salt stress induced significant changes in proteomic profiles of both cultivars

232   (SS and ST) and the responses to salt stress of ST were different to SS (Fig.2B). Meanwhile,

233   unsupervised cluster analysis showed a stability of replicates from each sample groups,

234   validating our treatments (Fig.2A). It further reach a consensus with PCA analysis that all

235   replicates with same treatments clustered together and observably separated with each other. We

236   further performed a two-factor (time point and strains) PCA analysis (Fig.2C,2D) on these

237   proteomic data sets to detected the response to salt stress at different time point. Focus on the

238   time points, we found that the most active responses were presented at 1h after imposing

239   (Fig.2C). Interestingly, the significant changes at 0h indicated that the basal level of proteomic

240   profile between SS and ST were different with each other (Fig.2D). Therefore, we proposed that

241   the basal level of some salt resistance-related protein of ST were different with SS, resulting in a

242   strong tolerance of ST to salt stress. Meanwhile, the ST can further make some responses under

243   salt stress at 1 h, which is superior than SS in response to salt stress.

244   Proteins located at chloroplast exhibited significant difference Salt tolerant cultivar (ST)
245   and Salt sensitive (SS) cultivar

246   To investigate the differences in proteome between SS and ST before salt stress, we compared

247   the proteomic profiles of ST and SS before treatment. Among   7663 identified proteins, only

248   protein in which the $\log_{1.5}$(fold change) > 1 or < -1 and the P value < 0.05 were identified as

249   differentially expressed proteins (DEPs; Fig.3A). Finally, 278 proteins matched the condition

250   were identified as DEPs (Fig.3A). Among 278 DEPs, there are 131 up-regulated proteins and

251   147 down-regulated proteins in ST compared to SS (Fig.3A). Stress-regulated genes, such as the

252   lipoxygenase (A0A3Q7ENA4), superoxide dismutase (Q7XAV2) and gamma aminobutyrate

253   transaminase 2 (A0A3Q7J2V8) were up-regulated in ST compared to their expression in SS,

254   suggesting that ST have more advantages in resistance to salt stress than SS. Meanwhile, we

255   found that the activity of two stress-related enzyme, SOD and PPO, were more active in ST than

256   that in SS, whereas the enzyme of other two stress-related enzyme, POD and PAO, did not

257   exhibited differences in activity in both SS and ST (Fig.3B, 3C). We further analyze the location

258   of these DEPs, the results indicated that most of differentially expressed proteins were located at

259   chloroplast, cytoplasm, nucleus, extracellular, plasma membrane and mitochondria,

260   demonstrating the proteins functioned at membrane and chloroplast contributed the differences

261   of resistance to salt stress between SS and ST (Fig.3E). It has been reported that various proteins

262   at chloroplast and membrane play key role in resistance to salt stress, including Superoxide

263   dismutase (Q7XAV2) and gamma aminobutyrate transaminase 2 (A0A3Q7J2V8; Arora et al.,

264   2002; Blokhina et al., 2003). Therefore, we concluded that the basal resistance of ST to salt

265   stress was higher than SS.

266   Gene enrichment analysis for DEGs from ST and SS

267   To study the differences of biological functions related to the diverse responses between SS and

268   ST, we performed a Gene ontology enrichment analysis on all 278 DEPs using TopGO (Fig4;

269   **Supplementary Table 3**). A total of 34 GO terms relevant to molecular progresses were

270   significantly enriched based on these 278 DEPs, including heme binding, tetrapyrrole binding,

271   peroxidase activity, oxidoreductase activity, peroxide acceptor, chitinase activity, antioxidant

272   activity, intramolecular lyase activity and catalytic activity (Fig. 4A). Various terms were

273   involved in the response to abiotic stress, such as peroxidase activity, oxidoreductase activity and

274   chitinase activity. In addition, among identified cellular component-related terms, 20 terms were

275   significantly enriched such as extracellular region, MCM complex, protein-DNA complex,

276   replisome, nuclear replication fork and nuclear replisome (Fig. 4A). Under salt stress, a bunch of

277   reactive oxygen species (ROS) could be produce in plants which cause damage to DNA and

278   membrane (GONG et al.,2013; Singh et al., 2016; Scott et al.,1987). Therefore, proteins having

279   DNA-, ROS- and membrane-related function could protect the DNA from damage, ensuring

280   normal biological processes. Finally, compared to SS, the results of GO enrichment analysis

281   further showed that 125 terms relevant to biological process category were significantly enriched

282   (P<0.05; Fig.4A). Remarkably, many stress-related biological processes were significantly

283   identified, including response to stress, reactive oxygen species metabolic process, hydrogen

284   peroxide metabolic process, response to stimulus, chitin metabolic process, cell wall

285   macromolecule catabolic process, response to oxidative stress, response to external stimulus and

286   cellular carbohydrate metabolic process (Fig. 4A), indicating that the resistance response to salt

287   stress were different between SS and ST. Moreover, the results of KOG analysis on these DEPs

288   showed that the expression of proteins which involved in signal transduction mechanisms,

289   energy production and conversion, carbohydrate transport and metabolism, amino acid transport

290   and metabolism, lipid transport and metabolism, secondary metabolites biosynthesis, transport

291   and catabolism were significantly altered in ST compared to SS (Fig. 4B). It has been proved that

292   amino acids such as proline and lipids such as glyceride play important functions in salt

293   resistance of plants (Ashraf et al., 2007; Butt et al., 2016; Huang et al., 2014). In parallel, signal

294   transduction also influence the plant salt resistance, such as hormone JA and ABA (Msanne et al.,

295   2011; MLLER et al., 2015; Zhu et al., 2010; LI et al., 2015; Huang et al., 2012; Guo et al., 2015).

296   Various studies have proved the important function of JA and ABA in strong resistance

297   responses of plants to salt stress (Ding et al., 2016; Me et al., 2016; ISLAM et al., 2017; Jiang et

298   al., 2016; LIU et al., 2015; GURMANI et al., 2013). In the pathways analysis, various

299   biosynthesis relevant to JA and ABA were significantly enriched, including α-linoleic acid

300   biosynthesis and steroid biosynthesis (Fig. 4A). Based on these results above, we proposed that

301   ST have more advantages in resisting salt stress compared to SS.

302   Proteins involved in various salt resistance-related pathways were altered in ST

303   Similarly, pathway enrichment analysis were also performed on all DEPs against KEGG

304   database. The results showed that there were 84 biosynthesis pathways were enriched (Fig.5A).

305   Among 84 pathways, 14 pathways were significantly enriched (Fig.5A; $P < 0.05$;

306   **Supplementary Table 4**), including Phenylpropanoid biosynthesis, Metabolic pathways,

307   Flavonoid biosynthesis, biosynthesis of secondary metabolites, DNA replication, Nitrogen

308   metabolism, Valine, leucine and isoleucine degradation and Zeatin biosynthesis (Fig.5A). It has

309   been reported that secondary metabolites played important roles in response to salt stress

310   (Hazman et al., 2015). The increase in content of betaine and sorbital could enhance the

311   resistance of various varieties to salt stress (Orthen et al., 1994; Kanayama et al., 2007; Gao et al.,

312   2000; Fu et al., 2011). Remarkably, all these metabolites-related pathways were significantly

313   reprogrammed in ST (Fig.5A), which may contribute to the advantages of ST in response to salt

314   stress. Based on these three function analyses, 112 DEPs which functioned in these terms were

315   identified (Fig.5B). In the expression pattern of these DEPs, two modules were constructed in the

316   heatmap (Fig.5B). Among these 112 DEPs, 76 were up-regulated in ST and clustered at the top

317   module which was labeled by pink square, whereas 36 proteins were down-regulated (Fig.5B).

318   Among highly up-regulated proteins, Superoxide dismutase (Q7XAV2), Lipoxygenase

319   (A0A3Q7ENA4) and various proteins relevant to chloroplast were involved in plant resistance

320   response to salt stress (Smirnoff, 2005; Andronis & Roubelakis- Angelakis, 2010; Schreiner &

321   Zozor, 1996). All these 76 up-regulated DEPs were further recognized as key proteins which

322   were important for the account for the mechanism of stronger salt resistance of ST than SS.

Responses of ST and SS were different under salt stress

324   To further clarify the advantage of ST in response to salt stress, we compared the proteomic

325   profiles of ST and SS after 1h imposing, which was observably separated with each other in PCA

326   plots. In ST1.vs.SS1 pairwise comparison, 169 proteins were identified as DEPs (Fig.6A).

327   Among 169 DEPs, 49 proteins were up-regulated in ST, whereas 120 proteins were up-regulated

328   in SS (Fig.6A). The expression level of various proteins functioned in response to salt stress

329   were higher than SS, including Photosystem II reaction center protein, non-specific lipid-transfer

330   protein 2-like, ABC transporter F family member 5, anion: sodium symporter and gamma

331   aminobutyrate transaminase 2. It has been proved that high expression level of lipid-transfer

332   protein could enhance the resistance of plants to salt stress, and such protein could also be

333   induced to up-regulated under salt stress (Li, 2004; Torres-Schumann et al., 1992). Interestingly,

334   all the DEPs were still mainly located at chloroplast, nucleus, cytoplasm, extracellular and

335   plasma membrane (Fig.6E). Remarkably, after 1h imposing, the enzyme activity of POD, PAO

336   and PPO were further activated in ST, whereas the activity of these three stress-related enzyme

337   were decreased in SS, which account for the advantage of ST in tolerance of salt challenge

338   (Table 1). However, the activity of SOD did not display significant changes in SS following salt

339   stress challenges (Table 1). Overall, all the 49 up-regulated DEPs were recognized as focus for

340   further analysis below to clarify the advantage of ST.

The advantage of ST in response to salt stress

342   To elucidate the advantage of ST in response to salt stress after 1h imposing, we further

343   performed function analysis. Compared to SS, all the up-regulated DEPs in ST after 1h imposing

344   were analyzed by Gene Ontology enrichment analysis (Fig. 7A, 7B, 7C). The results showed that

345   many significantly enriched molecular function-related terms were involved in salt stress, which

346   were helpful for plants to tolerate salt stress, including electron transporter, transferring electrons

347   within the cyclic electron transport, pathway of photosynthesis activity, structural constituent of

348   cytoskeleton, 4-aminobutyrate transaminase activity, ATP:ADP antiporter activity, heat shock

349   protein binding (Fig. 7B). Enzymes with ATPase activity perform important roles in plant

350   resistance to salt stress (Rangani et al., 2016). Meanwhile, aminobutyric acid is also an important

351   chemical component in plants response to salt stress, and 4-Aminobutanoate (GABA) can

352   alleviate the influences of salt stress on a variety of plants, including tomato, corn and wheat (Li

353   et al., 2016; Luo et al., 2012; Shi et al., 2010). For cellular component categorizes, gene

354   functioned in chloroplast-related terms were highly up-regulated in ST under salt stress,

355   including chloroplast, chloroplast part and chloroplast thylakoid (Fig. 7A). Under salt stress,

356   chloroplast could help plant scavenge the excessive accumulation of ROS and maintain the

357   normal growth of plants (Li et al.,2016; Lu et al.,2006). Furthermore, the significantly enriched

358   biological progresses relevant to salt stress were response to wounding, cellular carbohydrate

359   metabolic process, phloem development, cell wall biogenesis, response to stimulus and response

360   to hydrogen peroxide (Fig. 7C). The strengthened cell wall and carbohydrate metabolites are

361   important for plants as being challenged by salt stress (Pang et al., 2016; Dinneny et al., 2015;

362   Liu et al., 2015; Khan et al., 2016). Further pathway analysis on 169 DEPs showed an

363   overrepresentation of pathways relevant to resistance to salt stress, such as Alanine, aspartate and

364   glutamate metabolism, alpha-Linolenic acid metabolism, Linoleic acid metabolism, Peroxisome,

365   Steroid biosynthesis, Valine, leucine and isoleucine degradation (Fig.7D). Amino acid proline

366   could contribute the tolerance of plants to salt stress by functioning as a protective agent and

367   nitrogen source for cell structure and enzymes (Mansour et al., 1998; Butt et al., 2016).

368   Meanwhile, steroid biosynthesis could increase the content of ABA, which was widely identified

369   in plant response to salt stress and played important function in these processes (Albacete et al.,

370   2008, 2010). Remarkably, 21 proteins were identified from these important pathways and Go

371   function terms, including Lipoxygenase (Q84P54), gamma aminobutyrate transaminase 2

372   (A0A3Q7H8R6), Peroxidase (A0A3Q7EK65) and Glutamine synthetase (A0A3Q7J2V8)

373 (Fig.7E). Protein Peroxidase have been widely recognized as important role in salt resistance.

374 Therefore, we proposed that the advantage of ST in response to salt stress was mainly activation

375 of salt resistance-related metabolisms and alteration of cell structure.

Three key function proteins were important for the salt tolerance of ST

377 Overall, we found that three up-regulated proteins were shared in both ST.vs.SS and ST1.vs.SS1

378 pairwise comparisons, including A0A0C6G3Q8, A0A3Q7J2V8 and A0A3Q7IRF5 (Fig.8A;

379 Table2). Both A0A3Q7J2V8 and A0A3Q7IRF5 perform function at chloroplast, whereas

380 A0A0C6G3Q8 is located at plasma membrane (Table 2). Remarkably, all these 3 proteins up-

381 regulated 1.8~2.0-fold in ST.vs.SS pairwise comparison (Fig.8C, 8D, 8E). And their expression

382 in SS following 1h imposing was still almost 2.0-fold lower than ST after 1h imposing

383 (Fig.8C,8D, 8E). However, all of them could be induced to up-regulated in SS after 1h imposing

384 (Fig.8C, 8D,8 E). By function analysis of each proteins, we found that A0A0C6G3Q8 has Sterol

385 side chain reductase function, and A0A3Q7J2V8 is gamma aminobutyrate transaminase, whereas

386 A0A3Q7IRF5 involved in Starch synthase (Table2). Remarkably, up-regulation of

387 A0A0C6G3Q8 could promote the synthesis of Brassinolide in vivo, and the increase in content

388 of brassinolide may also contribute the plant resistance to salt stress and freezing injury, which

389 has been widely used in agriculture (Uday et al., 2010; Xia et al., 2009; Hua et al., 2011).

390 Additionally, up-regulation of A0A3Q7J2V8 encoding gamma aminobutyrate transaminase

391 promotes synthesis of GABA which has been widely used in filed to enhance plant resistance to

392 various abiotic stress (Li et al., 2016; Shi et al., 2010). Importantly, in the co-expression network

393 we further observed the hub role of these 3 proteins that all of them were located at the core of

394 the network and closely correlated with other up-regulated proteins which involved in resistance

395 to salt stress (Fig.8B). qRT-PCR analysis was further used for validation of these three key

396 proteins of ST in response to salt stress. The results showed there was no significant differences

397 in basal expression level of these three genes in both SS and ST cultivars. In contrast, dramatic

398 up-regulation of these genes was induced by 1 hour imposing in ST cultivars, whereas their

399   expression levels were not altered in SS after 1 hour imposing, leading a strong tolerance of ST

400   to salt stress (Fig 9). Therefore, we highlight the importance of A0A0C6G3Q8, A0A3Q7J2V8

401   and A0A3Q7IRF5 in the mechanism of resistance of ST to salt stress.

402   **DISCUSSION**

403   Salt stress seriously influences growth and development of tomato, which seriously restricts the

404   yield of tomato. With the development of researches, it has been showed that plants can resist

405   salt stress by various physiological changes. Among our both ST and SS cultivars, the activity of

406   SOD, PPO and POD was higher in ST cultivars, leading a strong tolerance to salt stress.

407   Subsequently, in this study, the changes in proteomic profiles of salt-tolerant and salt-sensitive

408   tomato varieties were compared. We found that salt-tolerant cultivars and salt-sensitive cultivars

409   had significantly different responses to salt stress.

410   Salt-tolerant cultivars could enhance the tolerance of plants to salt stress by changing the

411   expression of membrane structure-related proteins. Meanwhile, a variety of metabolic pathways

412   were altered between salt-tolerant and salt-sensitive cultivars. Compared with the Salt-sensitive

413   cultivars, there is a higher active pattern of metabolisms relevant to Nitrate and amino acids. And

414   under the challenge of salt stress, the salt-tolerant cultivars can further activated the

415   Brassinolides and GABA biosynthesis, which contribute to the resistance to salt stress.

416   Importantly, the higher expression of chloroplast-related protein in salt-tolerant cultivars

417   indicated that salt-tolerant cultivars had higher photosynthetic activity, which could

418   promotenormal growth of plants under salt stress. Additionally, Salt-tolerant plants have

419   advantages in scavenging reactive oxygen species produced by salt stress. A bunch of

420   antioxidant-related proteins were activated to up-regulated in Salt-tolerant cultivars, such as

421   peroxidase and Catalase isozyme. In-depth analysis, we identified three key proteins, which

422   highly expressed in Salt-tolerant cultivars and play key role in its strong resistance to salt stress,

423   including Sterol side chain reductase, gamma aminobutyrate transaminase and Starch synthase.

424   N-related metabolisms play important function in salt-tolerance of plants. A remarkable property

425   of salt-tolerant is that the proteins involved in N metabolism were highly up-regulated in ST. It

426   has been documented that high value of pH caused by salt stress will significantly influence the

427   N metabolism and absorption of plants (Groppa et al., 2008; Shan et al., 2016). The absorption of

428   NO_3^- by plants is mainly regulated by H^+/NO_3^- co-transporters, which requires high concentration

429   of protons around the channel as the transport energy (Dluzniewska et al., 2007). Under the salt

430   stress, the high pH environment around the roots leads to the shortage of protons around the

431   channels, which greatly restricts the assimilation or absorption of NO_3^- by the roots, and inhibits

432   the transport of NO_3^-, resulting in the decrease of the content of NO_3^- in the roots, thus

433   interfering the whole nitrogen metabolism pathway (Abouelsaad et al., 2016; Murtaza et al.,

434   2013; Shao et al., 2015). Our proteomic profiles of Salt-tolerant and Salt-sensitive cultivars

435   revealed that various proteins involved in N metabolism were highly up-regulated in Salt-tolerant

436   cultivars, in contrast these proteins were down-regulated in Salt-sensitive cultivars such as AMT

437   and NRT. Some studies have shown that plant roots transport NH_4^+ through AMT protein family,

438   and transport NO_3^- by NRT protein family. Under salt stress, the expression levels of OsNRT

439   and most members of OsAMT family in rice roots are increased, for the increase in absorption of

440   NO_3^- and NH_4^+ to make up for the deficiency of content in roots. These results suggested that the

441   N metabolism were not impaired in Salt-tolerant cultivars, which maintains the normal

442   physiological requirements of plants and enhance the tolerance of plants to salt stress. Therefore,

443   one of the advantages of Salt-tolerant cultivars was relative high levels of N metabolism.

444   Similarly, the salt-tolerant rice were also exhibited high level of N metabolism, which contribute

445   to its tolerance to salt stress (Lutts et al., 1999). Meanwhile, it also has been proved that the

446   amino acids-, carbohydrate- and flavonoids-related biosynthesis also performed important

447   function in the adaption of plants to salt stress.

448   Subsequently, our proteomic data showed a high expression level of genes relevant to GABA

449   and brassinolides biosynthesis. BR can improve plant resistance to biological and abiotic stress

450   (Uday et al., 2010;Hua et al., 2011). The regulation of BR on plant stress resistance was closely

451   related to the accumulation of H_2O_2 and the enhancement of the activity of antioxidant enzymes

452   (SOD, CAT, APX; Xia et al., 2009; Zhang et al., 2010). Exogenous BR can significantly

453   improve the growth and development of seedling plants under salt stress, reduce salt damage

454   index, and significantly increase the content of proline and soluble sugar in leaf cells and the

455   activity of antioxidant enzymes such as SOD, POD and CAT (Shang Qingmao et al., 2006), thus

456   improving the salt resistance of cucumber seedlings. Meanwhile, exogenous BR can improve the

457   salt tolerance of Arabidopsis thaliana (Hua et al., 2011). Under salt stress, exogenous EBR could

458   promote the activities of CAT and SOD enzymes in tomatoes, reduce the content of MDA in

459   cells, and improve the salt tolerance of tomatoes (Ahmmed et al., 2013). Gamma-aminobutyric

460   acid also plays an important role in plant salt tolerance, GABA can regulate cytoplasmic pH,

461   resist oxidative stress, interact with microorganisms, and defend insects as osmotic regulator and

462   signal molecule (Gilliham et al., 2016; Bouché et al., 2004). Appropriate concentration of GABA

463   can effectively promote seed germination and seedling growth under salt stress (Ziogas et al.,

464   2017). GABA can reduce oxidative damage by improving the antioxidant capacity of plants (Li

465   et al., 2016). At the same time, it can improve the content of osmotic regulation substances,

466   maintain osmotic balance (Li et al., 2010), inhibit photosynthetic pigment degradation, and

467   maintain photosystem II (PSII) activity. In addition, GABA can further regulate plant hormone

468   content (Shi et al., 2010) to improve plant salt tolerance. Therefore, high basal level of BRs and

469   GABA biosynthesis will be a new direction for breeding salt-tolerant cultivars.

470   Chloroplast were also important for the tolerance of plants to salt stress. Under abiotic stress, the

471   most vulnerable part is PSII (Wei et al., 2012). The photosynthetic rate decreased due to salt

472   stress, and salt stress would also reduce leaf turgor pressure and cause partial stomatal closure to

473   further inhibit photosynthesis (Chaves et al., 2009). Our data showed a high expression pattern of

474   proteins which involved in chloroplast membrane, demonstrating that the Salt-tolerant cultivars

475   have advantages in response to salt stress challenge. The salt-tolerant cultivars showed an

476   overrepresentation of chloroplast-related terms, which indicated that a bunch of proteins

477   functioned in chloroplast were purposefully highly expressed.   The reason is that plants need to

478   maintain the photosynthesis of plants at normal level, which providing adequate nutrient for

479   plants to enhance tolerance to salt stress. Additionally, various hormone-related biosynthesis

480   were also closely correlated with the chloroplast, such as ABA, JA and GA3. Meanwhile, plant

481   hormones play important roles in the signal transduction of plants in response to salt-stress,

482   including ABA and BRs (Yang et al., 2013; Dodd et al., 2009). Hormone synthesis is induced by

483   various abiotic stress, and their content varies with the degree of salt-stress. The changes in

484   content of hormone will initiate or regulate some physiological and biochemical processes

485   relevant to the response to abiotic stress. Researches have indicated that exogenous application

486   of ABA will enhance the tolerance of plants to salt stress (Dodd et al., 2009). We noted that both

487   biosynthesis of ABA and BRs were activated in Salt-tolerant cultivars, whereas these

488   biosynthesis exhibited an low level of activity in Salt-sensitive cultivars.

489   The ROS scavenging system is imperative for plant growth and development (Katalin et al.,

490   2011; Hajiboland et al., 2010). Under salt stress challenge, Plants always improve their salt-

491   tolerance by regulating the dynamic balance of reactive oxygen species (ROS), which is caused

492   by salt stress and results in damage to plants. In our research, we found that proteins involved in

493   ROS scavenging system was highly expressed in salt-tolerant tomato, suggesting that the salt-

494   tolerant cultivars have more advantages in scavenging of ROS, whose accumulation was caused

495   by salt stress,   than salt-sensitive cultivars. It has been proved that excessive accumulation of

496   reactive oxygen species will damage the integrity of the plant cell membrane system and

497   influence the plants development and growth (Smirnoff, 1993; Asada, 1999; Shigeoka et al.,

498   2002). Remarkably, excessive ROS accumulation will also cause damage to the ultrastructure of

499   chloroplast, which influences the photosynthetic system (Katalin et al., 2011). In parallel, it also

500   could inhibit the activity of photosynthesis-related enzyme, leading a decrease in efficient of

501   photosynthetic system (Katalin et al., 2011). In contrast, we have provided full evidences that the

502   chloroplast and photosynthesis of salt-tolerant cultivars were more active than that in salt-

503   sensitive cultivars. Active photosynthesis also could contribute to the scavenging of ROS by

504   activation of SOD (Arora et al., 2002; Andronis et al., 2010). Similar to our results, it has been

505   proved that there is less accumulation of ROS in the salt-tolerant cultivars, with high

506   photosynthesis in vivo. Therefore, we further recognized the highly active ROS scavenging

507   system as an important advantage of Salt-tolerant tomato as being challenges by salt stress.

508   Soil salinization has greatly restricted the production and development of agriculture. Breeding

509   salt-tolerant cultivars is still believed to be the most cost-effective strategy to deal with the

510   influence of salt stress on crops. However, the limited resources of salt-tolerant genes   restrict

511   the breeding of new tomato varieties. In our study, by comparative proteomic analysis, we found

512   that the advantages of Salt-tolerant tomato in response to salt stress were mainly high level of N-

513   and ABA-related biosynthesis, effective photosynthesis system and active ROS scavenging

514   system, leading a strong tolerance of tomato to salt stress. Additionally, high level of GABA and

515   Brassinolides biosynthesis also contribute the strong tolerance of tomato, and exogenous

516   application of both metabolites can be applied in yield. In parallel, three genes playing important

517   role in salt-resistance of Salt-tolerance tomato were further identified, including A0A3Q7J2V8,

518   A0A3Q7IRF5 and A0A0C6G3Q8, which can be used in future. Additionally, GABA and

519   Brassinolides were also involved in the plant resistance to salt stress. In summary, our results

520   provide mechanistic insights into the advantages of salt-tolerant cultivars vs. salt-sensitive

521   cultivars via comparative proteomic analysis.

522   **CONCLUSIONS**

523   Our results suggested that salt-tolerant cultivars have more advantages in resistance to salt stress

524   than that of salt-sensitive cultivars. The abundance and activity of antioxidant-related proteins,

525   such as SOD, PPO and POD, were higher in the salt-tolerant cultivars, leading effective

526   advantages in dealing with reactive oxygen species caused by salt stress. Meanwhile, higher

527   expression level of proteins relevant to Nitrate and amino acids metabolisms and photosynthetic

528   activity could guarantee a sufficient nutrient for the growth of salt-tolerant under salt stress.

529   Three key proteins functioned in Brassinolides and GABA biosynthesis, including Sterol side

530   chain reductase, gamma aminobutyrate transaminase and Starch synthase performed important

531   roles in regulation and activation of salt-resistance of salt-tolerant cultivars.

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536 **ADDITIONAL INFORMATION AND DECLARATIONS**

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540 **Competing Interests**

541 The authors declare that they have no competing interests

542 **Author Contributions**

543 • Qiang Wang conceived and designed the experiments, analyzed the data, contributed

544 reagents/materials/analysis tools, prepared figures and/or tables, approved the final draft.

545 • Baike Wang performed the experiments, contributed reagents/materials/analysis

546 tools,prepared figures and/or tables, approved the final draft.

547 • Huifang Liu, performed the experiments, contributed reagents/ analysis tools,prepared

548 figures and/or tables, approved the final draft.

549 • Hongwei Han analyzed the data, authored or reviewed drafts of the paper, approved the final

550 draft.

551 • Hongmei Zhuang analyzed the data, authored or reviewed drafts of the paper, approved the

552 final draft.

553 • Juan Wang analyzed the data, authored or reviewed drafts of the paper, approved the final

554 draft.

555 • Tao Yang analyzed the data, authored or reviewed drafts of the paper, approved the final

556 draft

557 • Hao Wang conceived and designed the experiments, performed the experiments, analyzed

558 the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and

559 approved the final draft.

560 • Yong Qin conceived and designed the experiments, performed the experiments, analyzed the

561   data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved

562   the final draft.

563 **Data Availability**

564 The datasets generated for this study can be found in the ProteomeXchange

565 (https://www.iprox.org) with the accession number PXD029130.

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870

Figure 1

Physiological changes between salt-tolerant(ST) and salt-sensitive(SS) cultivars

Basal content of carbohydrate (A), H_2O_2 (B) and total proteins (C) in SS and ST. significantly different at $P< 0.05$. Three biological replicates were performed.

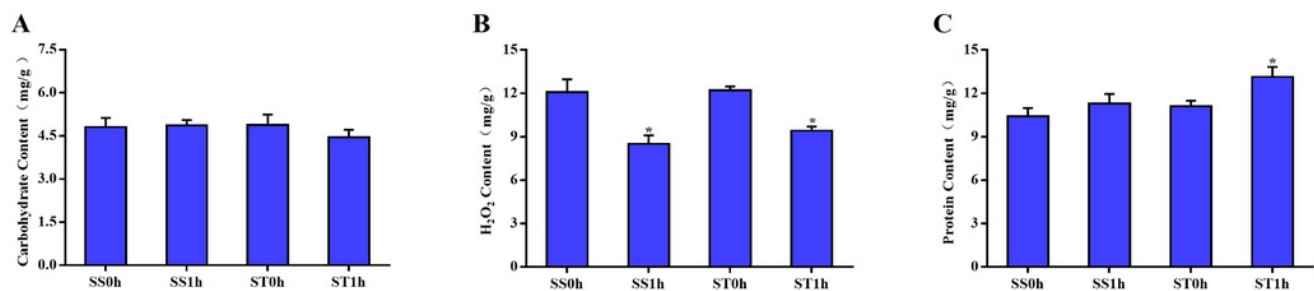



Figure 2

Landscape of proteomic profiles from all samples

A: Heat map showing the square of Pearson correlation coefficient between samples. The X and Y axes represent each sample. Coloring indicates the square of Pearson correlation coefficient (high: red; low: blue).B: Principle component analysis displayed the separation of all treatments, and stability of replicates with the same treatment. Each circle represents a sample, the circles labeled by same color were the samples with the same treatmentC, D: Two-factor Principle component analysis showing the main variation of proteome distributed in samples after 1h imposing, and comparisons of salt-tolerant (ST) and salt-sensitive (SS)cultivars.

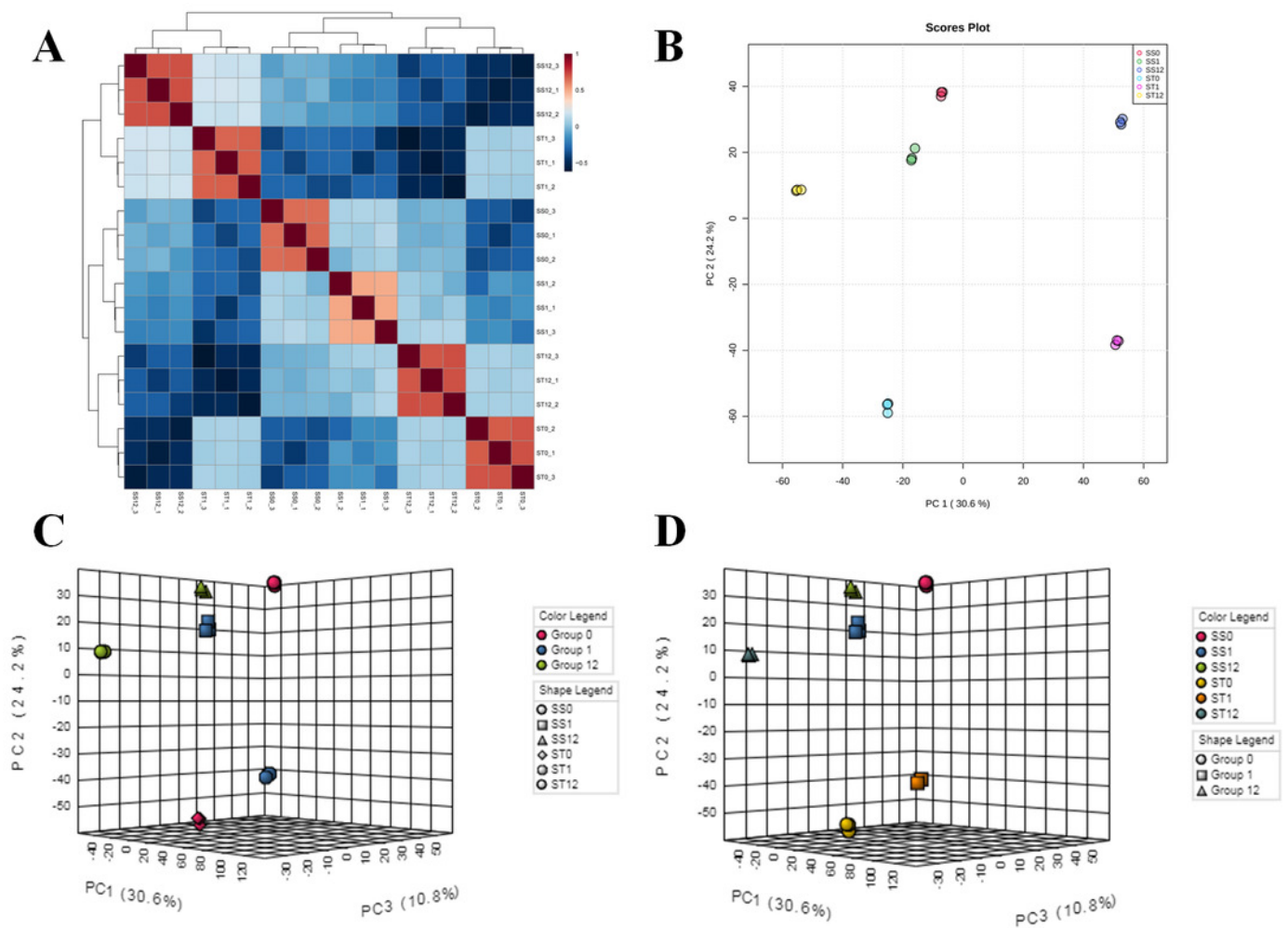



Figure 3

Location and variation of differentially expressed proteins between salt-tolerant (ST) and salt-sensitive (SS)cultivars

A: Volcano plots of differentially expressed proteins in SS and ST. Each plots represented a protein identified in SS and ST. The up-regulated proteins (Log1.5(Foldchange) >1.0; P< 0.05) were labeled by red, whereas the down-regulated proteins (Log1.5(Foldchange) < -1.0; P < 0.05) were shown in blue.B, C, D, E: Basal enzyme activity of Superoxide dismutase (SOD), polyphenol oxidase (PPO) , peroxidase (POD) and Polyamine oxidase(PAO)in SS and ST. Asterisk (*) indicates significantlydifferent at P< 0.05. Three biological replicates were performed.F: Location of differentially expressed proteins in ST.vs.SS pairwise comparison.

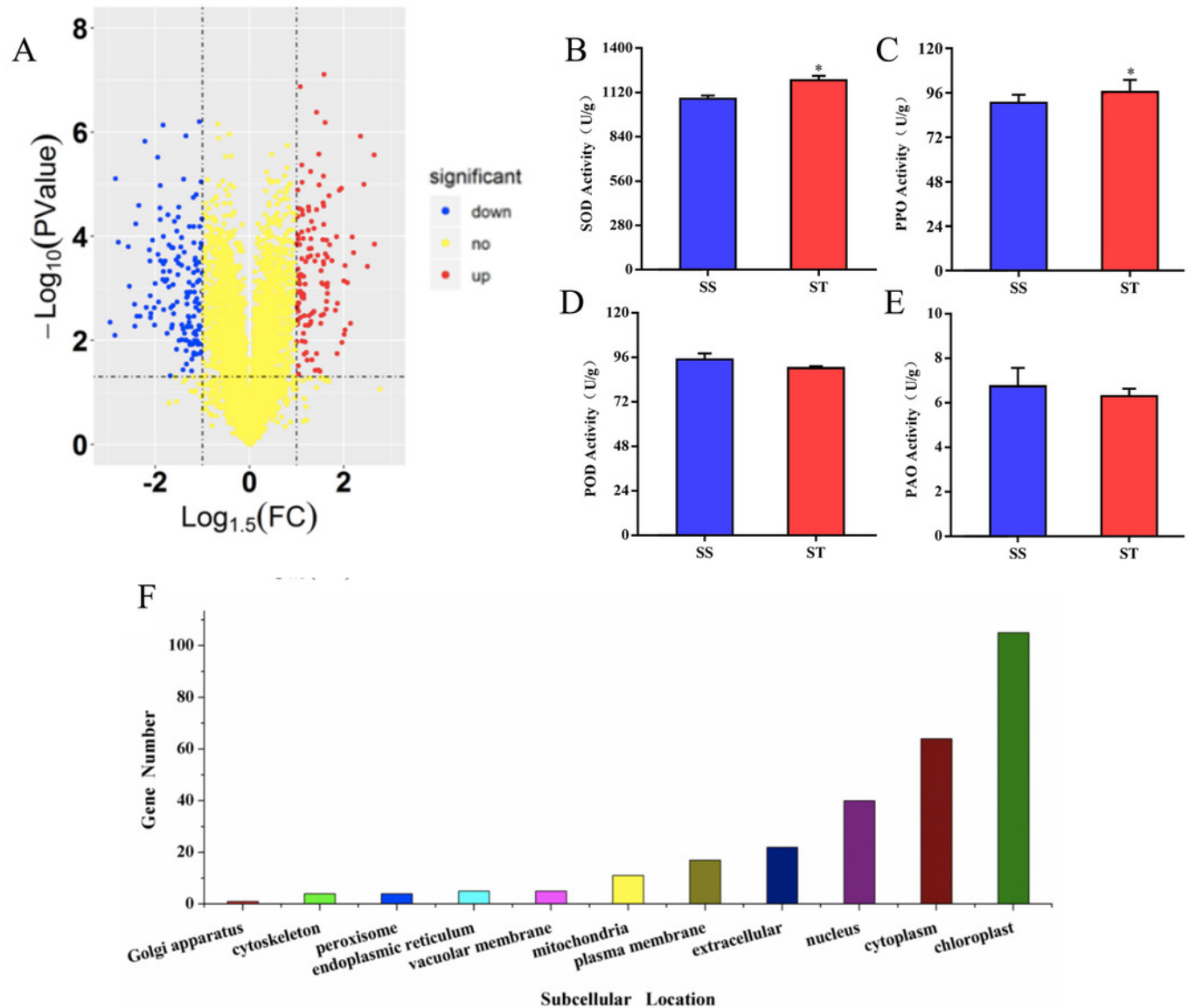
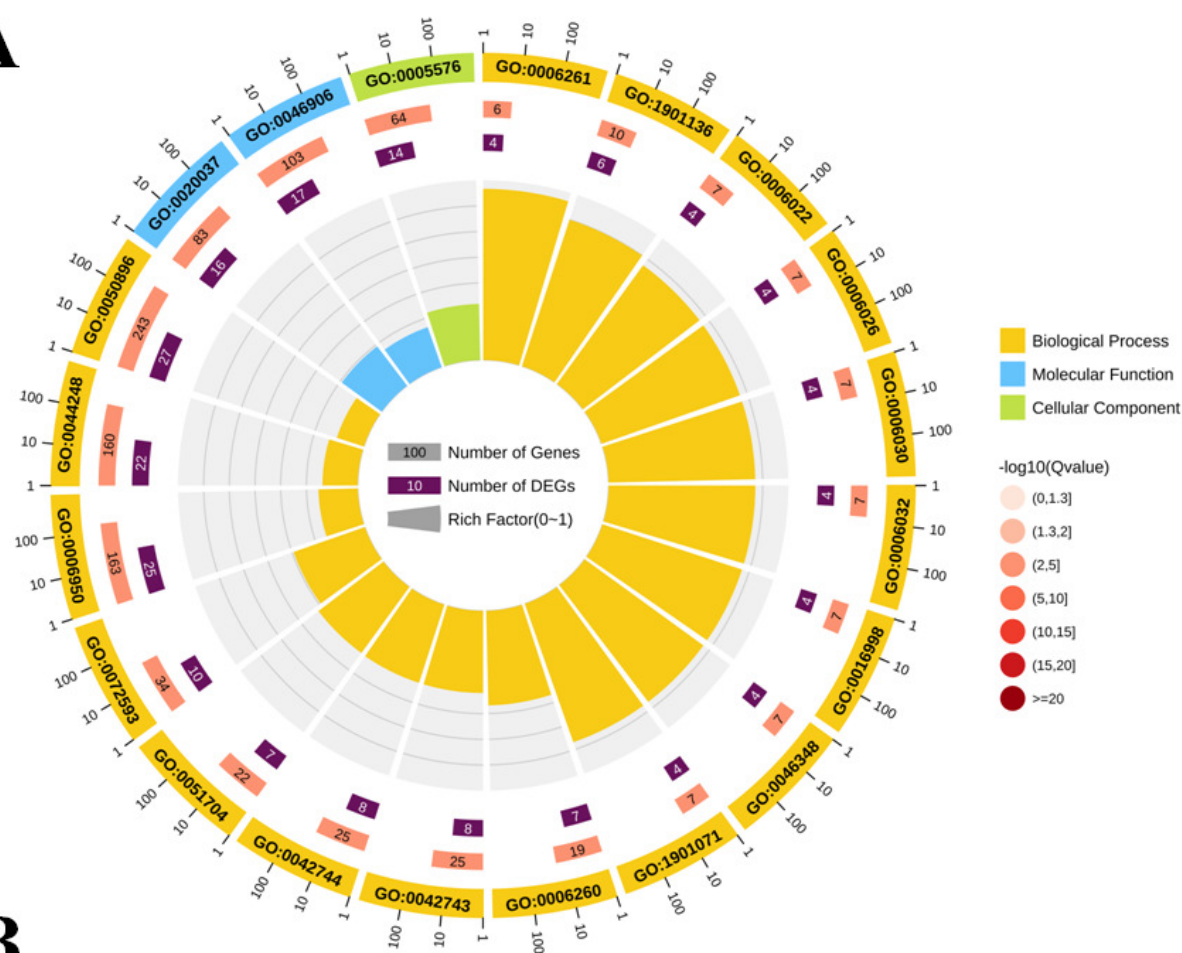



Figure 4

Function enrichment analysis on all differentially expressed proteins in salt-tolerant (ST) vs salt-sensitive(SS) cultivars

A: All differentially expressed proteins with Gene Ontology matches were assigned to three GO categories (cellular component, molecular function, and biological process). The terms relevant to biological progress was labeled by yellow square, where as the cellular component and molecular function were showed in blue and green squares, respectively. The number of proteins in each term was shown behind the square.B: Sequences with Gene Ontology matches were assigned to the KOG database and classified into 24 functional categories. The number of proteins in each categorizes was shown on the top the each column.

A

B

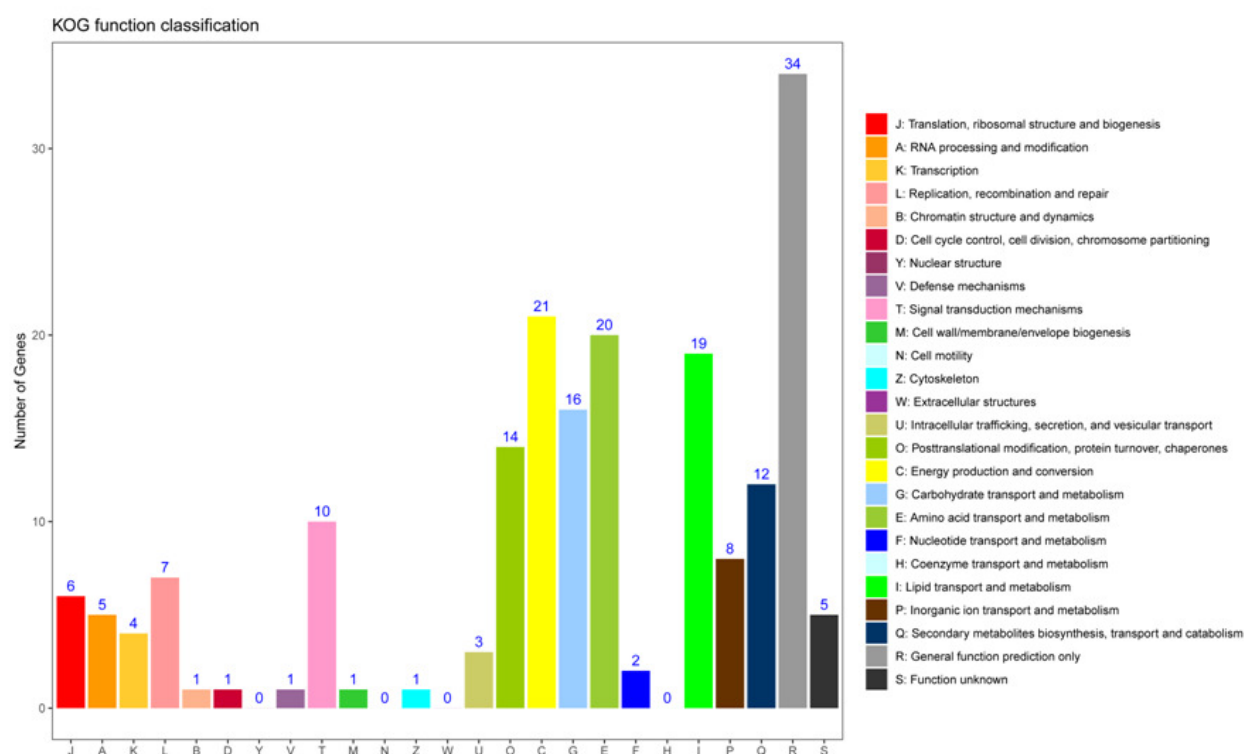



Figure 5

Pathway enrichment analysis of differentially expressed proteins in salt-tolerant(ST) vs salt-sensitive(SS) cultivars

A: Top 20 of Enriched pathways of differentially expressed proteins; The size of circle represented the number of proteins in the enriched pathway. And the significance were shown in different color (high: blue; low: red; P <0.05)B: Expression pattern of differentially expressed proteins in ST and SS. The highly expressed proteins in ST were shown in red, and the down-regulated proteins in ST were labeled by blue. Each cell represented a differentially expressed protein, and the columns were the samples of SS and ST.

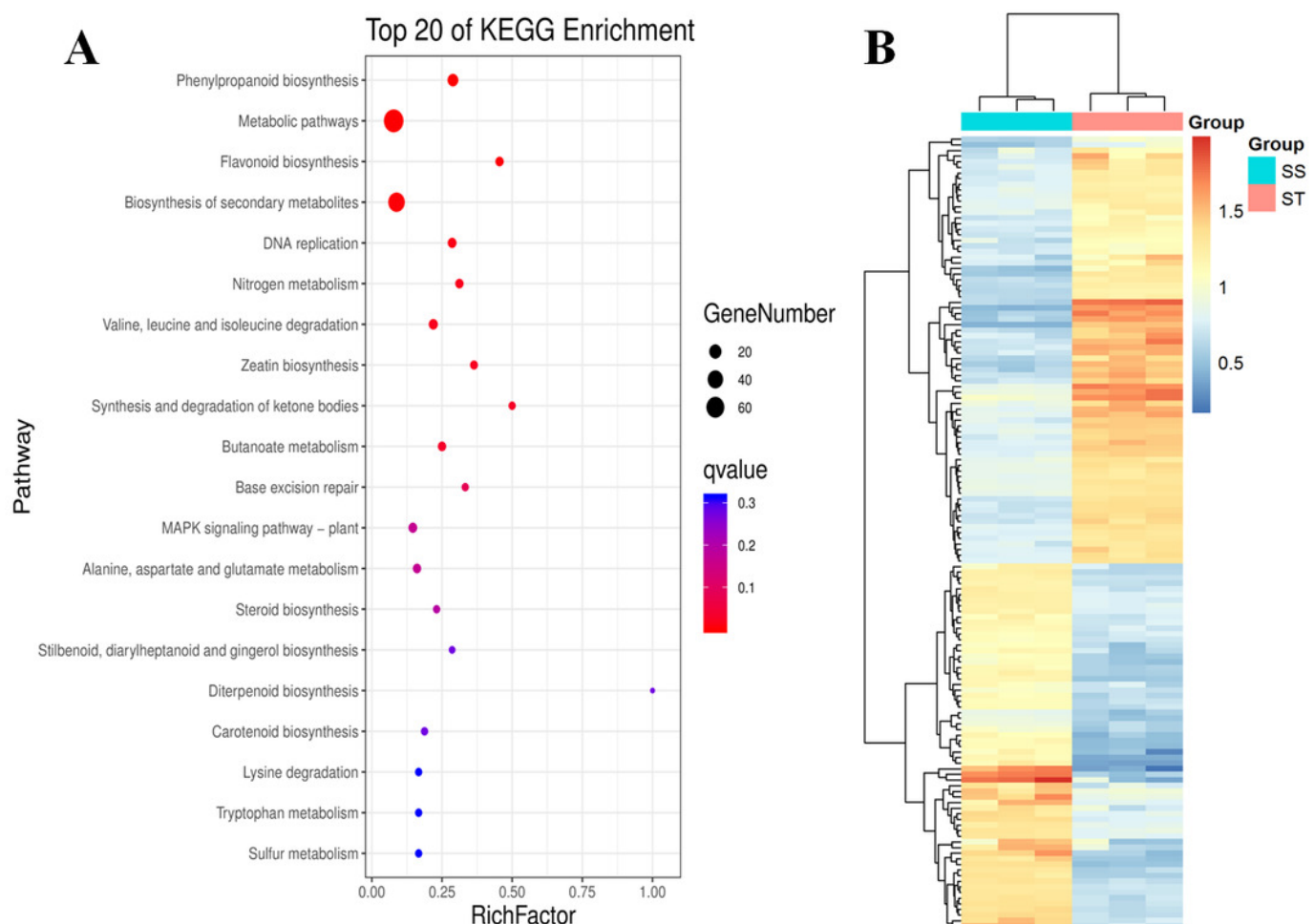



Figure 6

Location and variation of differentially Expressed proteins between salt-tolerant (ST) and salt-sensitive(SS) cultivars after 1h imposing

A: Volcano plots of differentially expressed proteins in SS1 and ST1. Each plots represented a protein identified in SS and ST. The up-regulated proteins (Log1.5(Foldchange) > 1.0; P < 0.05) were labeled by blue, whereas the down-regulated proteins (Log1.5(Foldchange) < -1.0; P < 0.05) were shown in red. The green points were the proteins without significant expression.B: Location of differentially expressed proteins in ST1.vs.SS1 pairwise comparison. Each column represented a structure of organisms.

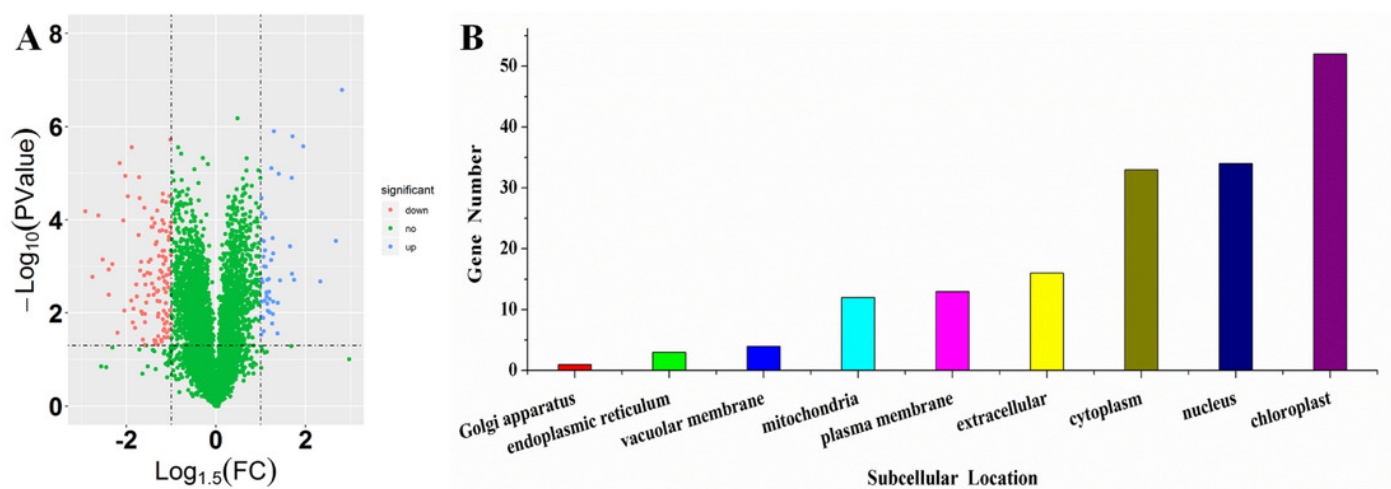



Figure 7

Function and pathway enrichment analysis on up-regulated proteins in salt-tolerant (ST) after 1h imposing

Top 20 of Enriched Gene ontology terms of differentially expressed proteins in ST after 1h imposing; The size of circle represented the number of proteins in the enriched Go terms. And the significance were shown in different color (high: blue; low: red; P <0.05); A: Cellular Component categorize; B: Molecular Function categorize; C: Biological Progress categorize.D: Top 20 of Enriched pathways of differentially expressed proteins in ST following 1h salt stress; The length of colum represented the number of proteins in the enriched pathway. And the significance were shown in different color (high: blue; low: black; P <0.05)E: Expression pattern of differentially expressed proteins in ST and SS following 1h salt stress. The highly expressed proteins which involved in response to salt stress in ST were shown in red, and the down-regulated proteins in ST were labeled by green. Each cell represented a differentially expressed protein, and the columns were the samples of SS and ST after 1h imposing.

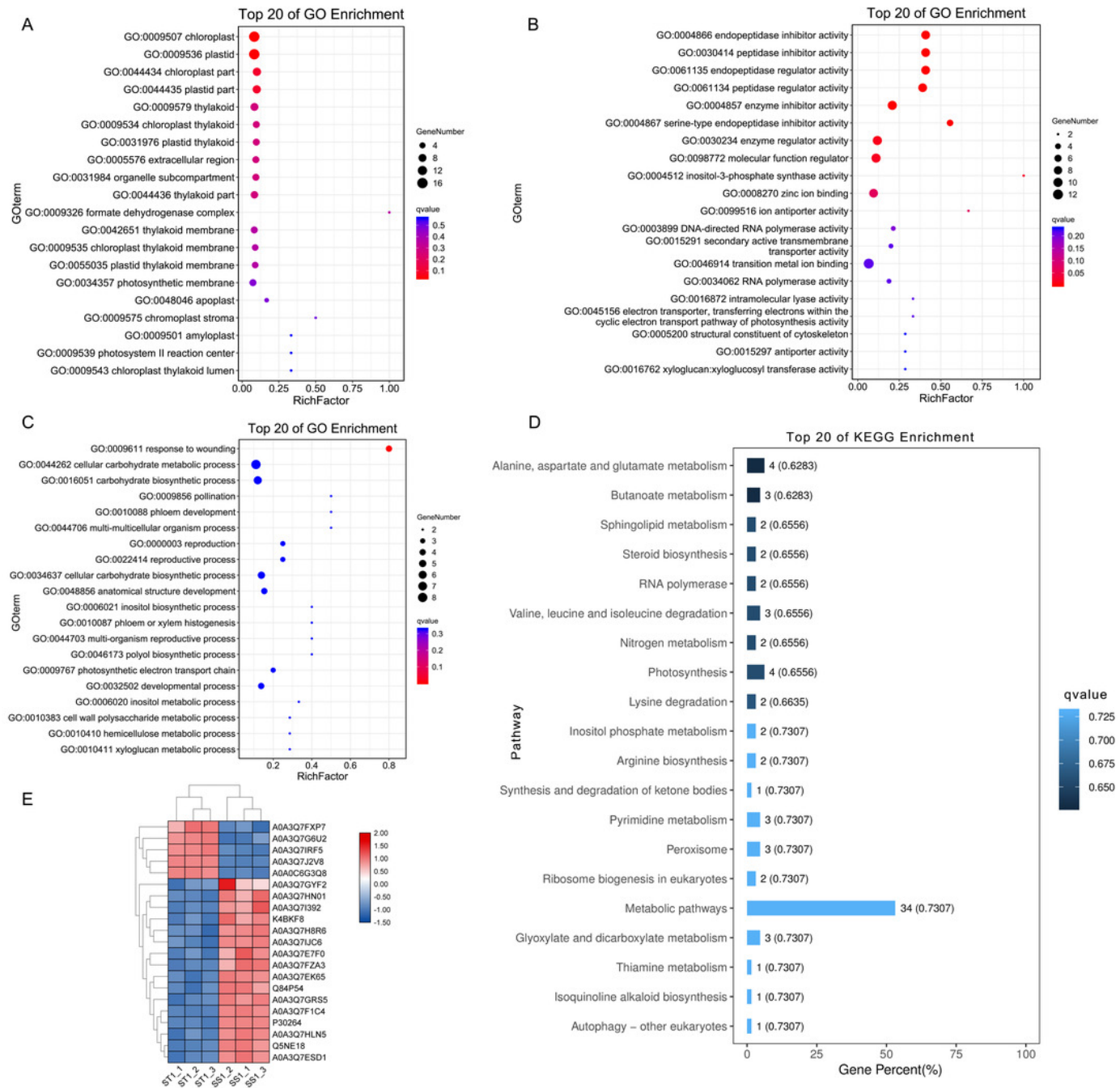



Figure 8

Three key proteins were involved in mechanism network of resistance of salt-tolerant (ST) to salt stress

A: Venn diagram of up-regulated proteins in ST.vs.SS and ST1.vs.SS1. Three up-regulated proteins were commonly identified in ST and ST1 after 1h imposing.B: Co-expression network of all up-regulated proteins which relevant to salt stress in ST. The key genes in the network were shown in red circles, indicating their hub status in response to salt stress. C, D, E: Relative expression of key proteins in each pairwise comparisons. The column with different color represented a singly pairwise comparison.

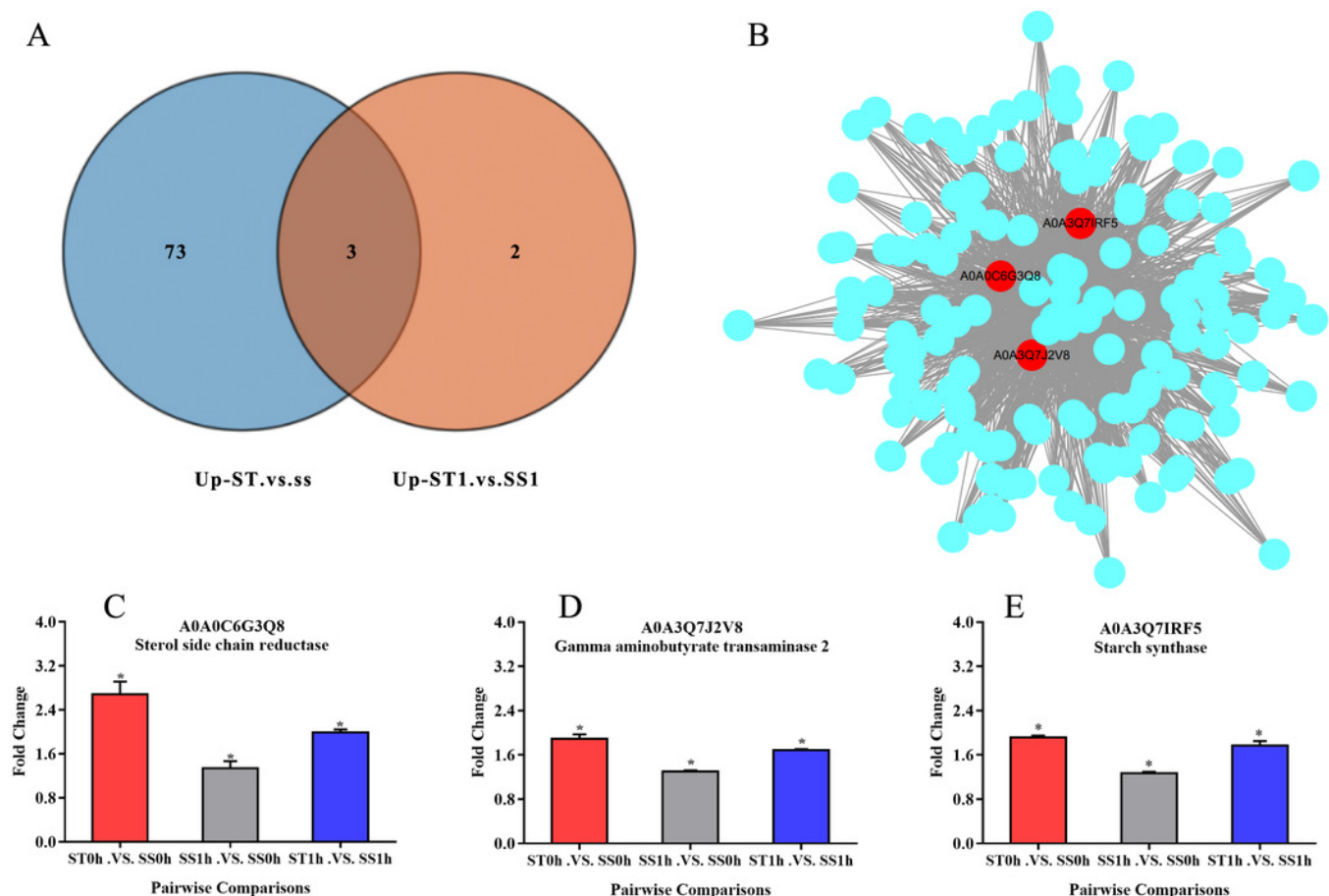
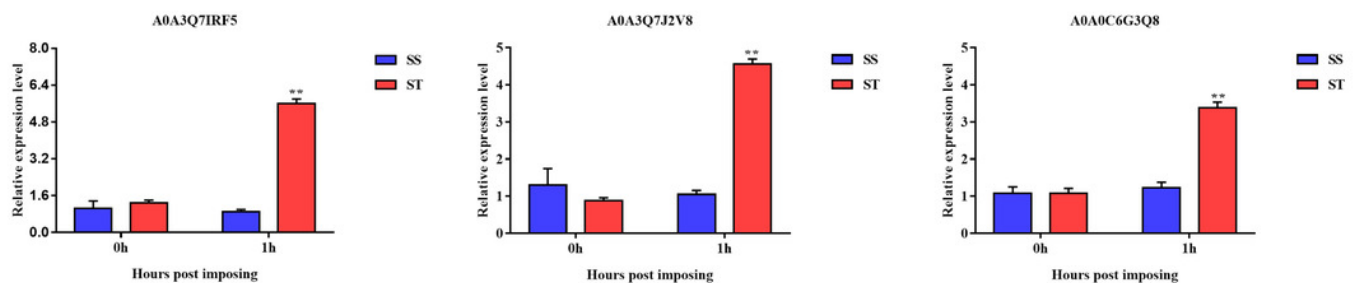



Figure 9

Validation of key genes expression using qRT-PCR

The relative expression level was represented by the $2^{-\Delta\Delta Ct}$ value, and the SS and ST were shown in green and red columns. Asterisk (**) indicates significantly different at P< 0.01.

Enzyme activity of salt-tolerant (ST)and salt-sensitive (SS) cultivars under salt stress challenge

significantly different at P< 0.05. Three biological replicates were performed.

Table 1. Enzyme activity of salt-tolerant (ST)and salt-sensitive (SS) cultivars under salt stress
challenge

	Enzyme Activity (U/g)			
	POD	PAO	PPO	SOD
SS0	94.99±3.15b	6.75±0.82b	90.53±4.43bc	1080.44±20.60c
SS1	49.65±1.14d	4.30±0.34c	81.28±5.33c	1045.90±9.24c
ST0	90.22±1.02c	6.30±0.33b	96.57±6.41b	1197.42±28.38a
ST1	145.98±1.77a	8.67±0.79a	110.52±4.65a	1117.93±13.24b

Note: significantly different at P< 0.05. Three biological replicates were performed.

Key proteins of salt-tolerant(ST) in response to salt stress

P < 0.05

Table 2. Key proteins of salt-tolerant(ST) in response to salt stress

Protein accession	Protein description	Subcellular Localization	KEGG
A0A0C6G3Q8	Sterol side chain reductase	plasma membrane	K09828
A0A3Q7J2V8	gamma aminobutyrate transaminase 2	chloroplast	K16871
A0A3Q7IRF5	Starch synthase, chloroplastic/amyloplastic	chloroplast	K13679

Note:P < 0.05