The full-length transcriptome of *Spartina alterniflora* reveals the complexity of high salt tolerance in monocotyledonous halophyte

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18 ABSTRACT

19 *Spartina alterniflora* (*Spartina*) is the only halophyte in the salt marsh. However, the
20 molecular basis of its high salt tolerance remains elusive. In this study, we used
21 PacBio full-length single molecule long-read sequencing and RNA-seq to elucidate
22 the transcriptome dynamics of high salt tolerance in *Spartina* by salt-gradient
23 experiments (0, 350, 500 and 800 mM NaCl). We systematically analyzed the gene
24 expression diversity and deciphered possible roles of ion transporters, protein kinases
25 and photosynthesis in salt tolerance. Moreover, the co-expression network analysis
26 revealed several hub genes in salt stress regulatory networks, including protein
27 kinases such as *SaOST1*, *SaCIPK10* and three *SaLRRs*. Furthermore, high salt stress
28 affected the gene expression of photosynthesis through down-regulation at the
29 transcription level and alternative splicing at the post-transcriptional level. In addition,
30 overexpression of two *Spartina* salt-tolerant genes *SaHSP70-I* and *SaAF2* in
31 *Arabidopsis* significantly promoted the salt tolerance of transgenic lines. Finally, we
32 built the SAPacBio website for visualizing the full-length transcriptome sequences,
33 transcription factors, ncRNAs, salt-tolerant genes, and alternative splicing events in

34 *Spartina*. Overall, this study sheds light on the high salt tolerance mechanisms of
 35 monocotyledonous-halophyte and demonstrates the potential of *Spartina* genes for
 36 engineering salt-tolerant plants.

37 Running title: *Spartina* transcriptome under salt stress

38 **Keywords:** *Spartina alterniflora*; high salt tolerance; ion transporter; protein kinase;
 39 regulatory hub genes; alternative splicing; single molecule real-time sequencing

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50 INTRODUCTION

51 Over 20% of the world's irrigated land suffers from soil salinization, and salt stress
 52 challenges the survival of plants through affecting their development and
 53 reproduction (Deinlein et al., 2014; Roy, Negrao, & Tester, 2014). However, only
 54 ~2% of terrestrial plants are salt-tolerant halophytes with strong ability to survive in
 55 concentrations of NaCl over 200 mM (Mangu et al., 2015). *Spartina alterniflora*
 56 (*Spartina*) is the only halophytic plant that can survive in highly saline environment
 57 of salt marshes (Subudhi & Baisakh, 2011). Intraspecific variation studies reveal that
 58 the strong saline environmental adaptation of *Spartina alterniflora* may be largely due
 59 to the greater ion selectivity to the uptake of potassium and excluding sodium
 60 (Bradley & Morris, 1991; Smart & Barko, 1980), which results in a lower Na^+/K^+
 61 ratio in *Spartina alterniflora* compared to the brackish water plant- *Spartina patens*
 62 (Hester, Mendelssohn, & McKee, 2001). Moreover, *Spartina alterniflora* also
 63 develops specialized salt glands to secret ions (Nestler, 1977; Subudhi & Baisakh,
 64 2011). Furthermore, cell membranes actively exclude salt out of cells to avoid the
 65 toxic effects of them inside cells (Subudhi & Baisakh, 2011). Therefore, *Spartina*

66 *alterniflora* exhibits a delicate ion exclusion system from physiology aspects to
67 survive hash saline environment and it is a good model plant to study high salt
68 tolerance. However, the molecular basis of salt tolerance in *Spartina alterniflora*
69 remains unexplored, and the post-transcriptional regulation of high salt tolerance has
70 not been characterized in plants.

71 Ion transporters play important roles in response to salt stress (J. K. Zhu, 2002,
72 2016). SOS1 (Salt Overlay Sensitive 1) is the most well-known Na^+/H^+ antiporter that
73 is used to extrude Na^+ from root cells into the soil and load Na^+ into the xylem for
74 long-distance transportation from roots to leaves via transpiration systems (Yang &
75 Guo, 2018; Y. Zhang et al., 2018; J. K. Zhu, 2002, 2016). Briefly, excessive salt stress
76 triggers cytoplasmic Ca^{2+} signaling, which is then sensed by SOS3 (J. K. Zhu, 2016).
77 SOS3 then interacts and activates the CBL-interacting protein kinase SOS2 (J. K. Zhu,
78 2016). The activated SOS2 then phosphorylates and activates the Na^+/H^+ antiporter
79 SOS1 (J. K. Zhu, 2016). HKT1 has an antagonistic function with SOS1 on
80 long-distance Na^+ transport and prevents Na^+ transport into leaves (J. K. Zhu, 2016).
81 Lower Na^+/K^+ ratio is very important in plant salt tolerance (G. Chen et al., 2015; Y.

Zhang et al., 2018; X. Zhu et al., 2018) and ion transporters from Shaker family are responsible for potassium uptake and transport (Barragan et al., 2012; Lacombe et al., 2000). AKT1 and AKT2 are two important potassium transporters from the Shaker family that function for both potassium influx and efflux (Y. Zhang et al., 2018). KAT2 and KAT1 interacted with each other to form inward K^+ channels (Nieves-Cordones et al., 2014). SKOR plays a role in delivering K^+ from the root stelar cells toward shoots (Gaymard et al., 1998). The K^+/H^+ antiporter NHX1 and NHX2 mediate the K^+ uptake from cytosol to vacuolar and regulate reservoir dynamics of K^+ (Barragan et al., 2012). However, none of these ion transporters has been characterized in *Spartina* and their regulatory roles in high salt tolerance are largely unexplored.

Protein phosphorylation is one important post-translational regulation mechanism and is regulated by protein kinases (Y. Ding et al., 2015). SOS2 represents one of the Ca^{2+} dependent protein kinases, including 10 SnRK2 and 25 SnRK3 in Arabidopsis (J. K. Zhu, 2016). In the SnRK2 family, SnRK2.10 is rapidly activated in Arabidopsis roots under salt stress (McLoughlin et al., 2012) and OST1 regulates cold stress by

98 phosphorylating C-repeat binding factors such as ICE1 under cold stress (Y. Ding et
99 al., 2018; Fujii, Verslues, & Zhu, 2011). In the SnRK3 family, the loss of Arabidopsis
100 SnRK3.16/CIPK1 and SnRK3.14/CIPK6 causes osmotic or salt stress sensitive
101 phenotype (D'Angelo et al., 2006; Tripathi, Parasuraman, Laxmi, & Chattopadhyay,
102 2009). In addition, the leucine-rich repeat receptor protein kinase (LRRs) was also
103 reported in response to environment stresses. For example, MIK2 is correlated with
104 mild salt tolerance by the natural variation study (Van der Does et al., 2017). The
105 overexpression of PnLRR-RLK27 from Antarctic moss confers salt tolerance (J.
106 Wang et al., 2017). On the contrary, Arabidopsis LRR-like kinase AtRPK1 and
107 OsRPK1 are negatively correlated with salt tolerance (Shi et al., 2014). However, it
108 remains elusive whether protein kinases involve in high salt stress associated
109 regulatory network.

110 Transcriptome sequencing has been widely adopted to explore the gene expression
111 dynamics in response to salt stress from different species (Bushman, Amundsen,
112 Warnke, Robins, & Johnson, 2016; Liu et al., 2019; Mutwakil et al., 2017; Qiu et al.,
113 2017). However, the transcriptome studies in high salt tolerance from halophyte are

relative limited and most of them mainly focus on dicotyledonous halophytes (Lee et al., 2013; Subudhi & Baisakh, 2011; Y. Zhang et al., 2008). Moreover, although the genome-wide transcriptome reprogramming under salt stress has been reported (Liu et al., 2019), the reprogramming under different salinity stress conditions remains unexplored. In addition, alternative splicing (AS) is one important post-transcriptional regulation that plays critical roles in eukaryotic pre-mRNA processing and contributes to diversifying the transcriptome and proteome (Deng & Cao, 2017). AS is affected by cold and heat stress in plants, and more importantly, ~10% of AS events are associated with salt stress in Arabidopsis (F. Ding et al., 2014; Srivastava, Lu, Zinta, Lang, & Zhu, 2018). Mutations of splicing factors such as SKIP, Sm-like protein 5, and SKB1 lead to the increase of AS events, especially intron retention (IR), under salt stress (Cui, Zhang, Ding, Ali, & Xiong, 2014; Feng et al., 2015; Z. Zhang et al., 2011), indicating the important function of AS in response to salt stress. However, how AS responds to salt stress in halophytes remains unclear. The main difficulty in analyzing transcriptome in *Spartina* is largely attributed to its complex genome with aneu-hexaploid ($2n = 6x = 62$) and the lack of high quality reference genome

130 (Renny-Byfield et al., 2010). Fortunately, the recently developed single-molecule
 131 long-read sequencing technology (SMRT) by the Pacific Biosciences (PacBio)
 132 platform can sequence full-length mRNA isoforms without *de novo* assembly, which
 133 has been widely adopted to profile full-length transcripts in *Salvia miltiorrhiza*,
 134 *Sorghum bicolor*, *Phyllostachys edulis*, *Drynaria roosii*, maize, wheat and strawberry
 135 (Abdel-Ghany et al., 2016; An, Cao, Li, Humbeck, & Wang, 2018; Dong et al., 2015;
 136 Y. Li, Dai, Hu, Liu, & Kang, 2017; Sun et al., 2018; B. Wang et al., 2016; T. Wang et
 137 al., 2017; Xu et al., 2015). These pioneering studies provide elegant solutions for the
 138 study of biological mechanisms in poorly-annotated species.

139 In this study, we used PacBio SMRT sequencing and RNA-seq to get insight into
 140 the high salt tolerance mechanism of *Spartina alterniflora*. Full-length reference
 141 transcript sequences of *Spartina* were obtained. We uncovered the crucial role of ion
 142 transporters associated with the high salt tolerance and identified hub genes in the
 143 high salt tolerant regulatory network. The involvement of alternative splicing in high
 144 salt tolerance was also documented. Furthermore, candidate transcripts for salt
 145 tolerance of *Spartina* were functionally analyzed in Arabidopsis. We also built the

SAPacBio website (<http://plantpolya.org/SAPacBio/>) for visualization and query of full-length transcriptome sequences in *Spartina*. Overall, our results provide new insights into the high salt tolerance regulatory mechanism of halophytes and demonstrate the great potential of *Spartina* to provide genetic resources for engineering salt-tolerant plants.

MATERIALS AND METHODS

Plant Materials

Spartina alterniflora seeds were collected in Ganyu County (E119°16'; N34°46'), Jiangsu Province, China. *Spartina* seedlings were grown on half-strength Hoagland liquid medium for 6 weeks in a growth chamber under a 16-hour light (80 $\mu\text{mol m}^{-2}\text{s}^{-1}$)/8-hour dark regime at 26°C (day) and 16°C (night). Plants with similar vigor were selected and salinized for 24 hours, in triplicate, at different NaCl concentrations (0, 350, 500, and 800 mM). These concentrations were selected to mimic non-salt-stressed conditions, low, medium, and high-salinity stress, since the lethal salt concentration for *Spartina* germination is ~1000 mM NaCl (Subudhi & Baisakh,

2011), and the average salinity level of the sea is ~599 mM (3.5%) NaCl. Total RNAs were isolated (Tiangen, Cat.DP441) and high-quality samples (RNA integrity number > 8) were used to prepare libraries for experimental validation.

Library construction and sequencing

Pacbio SMRT and RNA-seq (Levin et al., 2010) were performed using whole seedlings grown at different salt concentrations (0, 350, 500, and 800 mM NaCl), as described previously (T. Wang et al., 2017). These libraries were sequenced on the PacBio RSII platform (Iso-seq) and the HiSeq 2500 sequencing platform (RNA-seq, paired-ends 125 bp).

Bioinformatics analysis

Spartina full-length non-chimeric (FLNC) transcripts were identified from PacBio SMRT reads using the PacBio's SMRT Analysis pipeline (v2.3.0) (Gordon et al., 2015), and were further corrected by proovread (Hackl, Hedrich, Schultz, & Forster, 2014) using Illumina RNA-seq data. These FLNC transcripts were clustered into

178 unique transcript clusters by a two-rounds clustering incorporating CD-HIT-EST
 179 (v4.6) (Fu, Niu, Zhu, Wu, & Li, 2012) and GMAP (v2016-06-09) (T. D. Wu &
 180 Watanabe, 2005), and the longest isoform was defined as the unigene for each cluster.

181 The 5' UTR, open reading frame (ORF), and 3' UTR of each FLNC transcript
 182 were obtained using TransDecode (v3.0.1, <https://transdecoder.github.io/>). Functional
 183 annotations were generated using Blast2GO (blastx 2.2.26+) (Conesa et al., 2005)
 184 with the National Center for Biotechnology Information's (NCBI) non-redundant
 185 database (e-value < 1e-5). Transcript datasets of *Oryza sativa* (IRSGP1.0) and
 186 *Arabidopsis thaliana* (TAIR10) were downloaded from EnsemblPlants (release34,
 187 <http://plants.ensembl.org>). All isoforms of *Spartina* were aligned to these datasets
 188 using blastn (e-value < 1e-5).

189 Gene ontology (GO) enrichment analysis (p-value < 0.05) was performed by
 190 BiNGO (Maere, Heymans, & Kuiper, 2005). Non-coding RNAs (ncRNAs) were
 191 predicted using CPAT (coding score < 0.298) (L. Wang et al., 2013) and the plant
 192 non-coding RNA database (PNRD) (e-value < 1e-5) (Yi, Zhang, Ling, Xu, & Su,
 193 2015). Transcription factors were identified using the Plant Transcription Factor

194 Database (PTFD, v4.0) with default parameters (Jin et al., 2017).

195

196 **Co-expression network analysis and alternative splicing**

197 Differentially expressed (DE) unigenes were identified from RNA-seq data by RSEM

198 (v1.2.25) combined with bowtie2 (v2.2.9) and EBSeq (v1.20.0) (Leng et al., 2013; B.

199 Li & Dewey, 2011) based on the following criteria: false discovery rate (FDR)<0.05,

200 and |fold change|≥2. All DE genes were used to construct the co-expression network

201 by WGCNA (v1.66) (Langfelder & Horvath, 2008). The GO enrichment analysis of

202 genes in each module was conducted by topGO (V2.32.0)(Alexa, Rahnenfuhrer, &

203 Lengauer, 2006). Hub genes from the salt stress network were identified using

204 Cytoscape plugin cytoHubba and ranked according to the maximal clique centrality

205 (MCC) scores (Chin et al., 2014). AS events were identified using AStrap (Ji et al.,

206 2018) and differentially expressed alternative splicing events (DEAS) were also

207 characterized by using RNA-seq data (see Supplemental Methods).

208

209

210 **Experimental validation**

211 RT-PCR and qRT-PCR experiments were conducted as described in a previous study
 212 (T. Wang et al., 2017). Primers used are listed in Table S1. Results from RT-PCR
 213 were visualized in 1% agarose gels. Coding sequences of *SaAF2* and *SaHSP70-I*
 214 (without stop codons) were cloned into modified pCambia3301 constructs
 215 (*pACT2::gene-GFP*), and transformed into the gene slicing suppression *rdr6-11*
 216 Arabidopsis mutants (Peragine, Yoshikawa, Wu, Albrecht, & Poethig, 2004). Two
 217 independent T₃ transgenic lines were grown on plates containing half-strength
 218 Murashige and Skoog medium (1/2 MS) for 10 days under different salinity stress
 219 conditions (0, 120, and 150 mM NaCl). The primary root length was measured using
 220 ImageJ (Schneider, Rasband, & Eliceiri, 2012).

221

222 **Availability and accession numbers**

223 Datasets from Illumina HiSeq 2500 and PacBio SMRT sequencing have been
 224 deposited at the NCBI website under the Bioproject accession number PRJNA413596.
 225 The SAPacBio website was built to query and visualize full-length isoforms, DE

226 genes and AS in *Spartina* (<http://plantpolya.org/SAPacBio/>).

227 RESULTS

228 Characterization of the full-length transcriptome in *Spartina alterniflora*

229 We used PacBio SMRT sequencing to obtain full-length reference transcripts of
230 high-confidence in *Spartina* to avoid potential bias attributed to its complex
231 aneu-hexaploid genome ($2n = 6x = 62$) (Bedre, Mangu, Srivastava, Sanchez, &
232 Baisakh, 2016). An overview of the experimental procedure is illustrated in Fig. 1.
233 Briefly, 6-week-old whole *Spartina* plants were treated with 0, 350, 500, and 800 mM
234 NaCl for 24 hours to simulate non-salt stressed conditions, low, medium, and high
235 salt-stressed conditions. Total RNA was isolated from each salt stress condition and
236 mixed equally to prepare three different size-selected libraries (1-2 kb, 2-3 kb,
237 and > 3 kb). Seven cells were sequenced on the PacBio RSII real-time (RT)
238 sequencing platform. The length distribution of reads of inserts (ROIs) in each library
239 is consistent with the selected size (Fig. 2a and Table S2).

240 After the quality control by SMRT analysis (Gordon et al., 2015) and further
241 correction with ~250 millions Illumina RNA-seq reads by proovread (Hackl et al.,

2014), a total of 179,194 FLNC reads (including a poly(A) tail, 5' UTR and 3' UTR) were obtained, 90,587 of which are non-redundant FLNC reads (Table S2). Over 90% of FLNC transcripts are ORF-containing transcripts with an average coding sequence (CDS) length of 1601 bp. These FLNC transcripts cover a wide variety of functional annotations such as catalytic activity, metabolic processes, and transporter activity (Fig. S1). These transcripts were further grouped into 24,670 unique transcript clusters (unigenes). Among them, 55.72% contained multiple isoforms (Fig. 2b), the percentage of which is comparable to that from previous studies (M. Wang et al., 2018; T. Wang et al., 2017). The number of isoforms in each cluster is correlated with their expression; single isoform-containing clusters tend to have lower expression levels than clusters with multiple isoforms (Fig. S2). Additionally, 3494 ncRNAs (Table S3) were identified. Furthermore, we used the plant transcription factor database (Jin *et al.*, 2017) to identify transcription factors (TFs) in *Spartina*. In total, 4997 transcripts from 1323 unigenes were identified as encoding functional domains of TF families (Tables S4-5).

We further compared FLNC transcripts from PacBio with those obtained from

258 RNA-seq data (Bedre et al., 2016). The average length of FLNC transcripts (2405 bp)
259 is significantly longer than that from RNA-seq data (324 bp) (Bedre et al., 2016).
260 Over 73% of RNA-seq contigs can be mapped to FLNC transcripts, while 64,755
261 FLNC transcripts are newly discovered and not present in RNA-seq data (Fig. 2c).
262 Eight FLNC transcripts were randomly selected and all were further validated by
263 RT-PCR (Fig. S3). Overall, our PacBio FLNC transcripts provide qualified full-length
264 reference sequences for comprehensive profiling of the *Spartina* transcriptome.

265

266 **Genome-wide comparison of *Spartina* full-length transcriptome to other species**

267 As *Spartina* is a monocot halophyte (Subudhi & Baisakh, 2011), we examined
268 whether its transcripts are homologous to other plants. To this end, FLNC transcripts
269 of *Spartina* were mapped to Arabidopsis and rice. Less than 9% of transcripts were
270 mapped to Arabidopsis, while over 80% are highly similar to transcripts in rice (Fig.
271 2d). These results indicated that *Spartina* is highly homologous to the
272 monocotyledonous-crop rice, and thus has the potential to provide genetic resources
273 for engineering salt-tolerant rice. To understand the potential difference between

274 *Spartina* and other plant species, we conducted a comparison of GO term percentages
275 that was previously used to compare *Arabidopsis thaliana* and *Thellungiella*
276 *salsuginea* (H. J. Wu et al., 2012). GO terms were mapped to 19,093 unigenes in
277 *Spartina* using Blast2GO (Conesa et al., 2005), and were assigned to 30,821
278 annotated genes in *Arabidopsis thaliana* and 30,241 annotated genes in *Oryza sativa*
279 using AgriGO (Tian et al., 2017). Detailed analysis revealed that the proportion of
280 genes in the “membrane” (35.8% for *Spartina*, 27% for *Arabidopsis*, 9.5% for rice,
281 with Fisher’s exact test p-value=1.94e-95 for the comparison between *Spartina* and
282 *Arabidopsis*, and p-value=0 for *Spartina* and rice), “localization” (13.4% for *Spartina*,
283 8.8% for *Arabidopsis*, 7.3% for rice, with Fisher’s test p-value=4.70e-56 for *Spartina*
284 and *Arabidopsis*, and p-value=6.18e-108 for *Spartina* and rice) and “transporter
285 activity” (6.6% for *Spartina*, 4.6% for *Arabidopsis*, 4.8% for rice, with Fisher’s test
286 p-value=2.06e-20 for *Spartina* and *Arabidopsis*, and p-value=7.19e-17 for *Spartina*
287 and rice) categories were significantly increased in *Spartina* compared to both rice
288 and *Arabidopsis* (**Figs. 3c-d**). As transporters are mainly membrane proteins and
289 modulate cellular localization, the increasing percentage of transporter coding genes

might contribute to the high salt tolerance phenotype in *Spartina*. Furthermore, *Spartina* transcripts differ from transcripts in other plant species, suggesting that these transcripts might be specifically expressed in *Spartina* and play unique roles in salt adaptation (Table S6).

Genome-wide dynamics of gene expression profile under salt stress in *Spartina*

To identify salt-responsive transcripts and explore salt tolerance mechanisms in *Spartina*, twelve RNA-seq libraries were constructed from plants grown under four salt stress gradients with three biological replicates. RNA-seq samples from the same conditions were clustered together, indicating the high reproducibility of our RNA-seq data (Fig. S4). A total of 3875 unigenes are differentially expressed ($FDR < 0.05$ and $|\log_2FC| \geq 1$) among the salt-gradient experiments (Table S7). Moreover, 5108 FLNC transcripts, corresponding to 3375 unigenes, are differentially expressed between salinity stresses and control conditions. The number of DE transcripts increases with the salinity gradient (Fig. S5a). Moreover, the number of up-regulated transcripts is greater than that of down-regulated transcripts, particularly under high

salt stress (Fig. **S5b-c**). The same trend was observed for DE unigenes (Figs. **4a**, **S5d-f**), indicating that salt stress significantly affects the expression of *Spartina* unigenes.

Next, high salt stress associated unigenes (DE unigenes between 800 mM NaCl and control) were used to identify enriched pathways. Up-regulated unigenes are enriched in pathways including ion transport, response to stress, and amino acid metabolic processes (Fig. **4b**), while photosynthesis and translation are significantly enriched in down-regulated unigenes (Fig. **4c**). Importantly, these pathways are crucial components of salt stress-responsive mechanisms in plants (Deinlein et al., 2014; Munns & Tester, 2008; Soni et al., 2015), suggesting that high salt stress greatly affects the gene expression of salt-responsive pathways in *Spartina*.

To validate high salinity stress-associated unigenes, 15 DE unigenes were randomly selected for qRT-PCR experiments. The relative transcript levels measured by qRT-PCR and RNA-seq correlated significantly (Pearson correlation = 0.713, p-value = 3.908e-08, Fig. **4d**). In addition, 10 DE unigenes were randomly selected for RT-PCR experiments and results confirmed the gene expression pattern from

322 RNA-seq (Fig. S6).

323

324 **Co-expression network analysis reveals regulatory hub genes under high salt**
 325 **stress**

326 To unveil the interrelationships among high-salt-stress responsive genes, we
 327 performed the weighted gene co-expression networks analysis (WGCNA) (Liu et al.,
 328 2019) with all 3875 DE unigenes from varying degrees of salt stress (Table S7). The
 329 co-expression network was built and DE unigenes were classified into five modules
 330 with hierarchical clustering. Different colors (blue, brown, green, turquoise, yellow)
 331 were used to represent distinct modules.

332 The largest module turquoise contains 2380 DE unigenes, showing an increasing
 333 expression trend especially under high salt stress (800 mM NaCl) (Table S8). We next
 334 performed gene ontology analysis to decipher potential functions of DE unigenes in
 335 the turquoise module. These genes are significantly enriched in “cellular homeostasis”,
 336 “chemical homeostasis” and “carbohydrate homeostasis” (Table S8). Specially, we
 337 observed that ion homeostasis processes such as metal ion homeostasis, cellular ion

homeostasis, cellular metal ion homeostasis and transition metal ion homeostasis are enriched in DE genes of the turquoise module (Table S8). Consistently, transporters response to these homeostasis processes are also over-represented, which is further supported by the enrichment of “plasma membrane” under cellular components category and “transporter activity” under molecular function category (Table S8). These results suggested that transporters are involved in response to high salt stress.

The second largest module (blue) contained 1261 DE unigenes and showed a decreasing expression trends along with salt stress gradient, which was opposite with the turquoise module (Table S8). DE genes of the blue module are mainly enriched in “translation” and “photosynthesis” (Table S8). The module brown (97 DE genes) was induced under salt stress of 350 mM and 500 mM NaCl and is enriched in “small molecule metabolic process”, “methylation” and “amino acid metabolic process” (Table S8).

To uncover potential key regulators under high salt stress, we identified top-ten hub genes from each module. Several genes related to environmental stress regulation were identified (Fig. 4e-f). In the turquoise up-regulation module (Fig. 4e), *OST1*

354 (*Cluster17805*, also known as *SnRK2.6* or *SnRK2E*) encodes an ABA activated
 355 protein kinase, playing vital roles in the ionic osmotic stress by regulating the
 356 stomatal closure (Boudsocq, Barbier-Brygoo, & Lauriere, 2004; Fujii et al., 2011).
 357 *CHIA* (*Cluster32181*) encodes a chitinase, which was reported not expressed at
 358 normal conditions but exclusively expressed under environmental stresses especially
 359 salt and wound stress in Arabidopsis (Takenaka, Nakano, Tamoi, Sakuda, &
 360 Fukamizo, 2009). *CIPK10* (*Cluster17512*, also known as *PKS2*, *SIP1*, and *SnRK3.8*)
 361 encodes a CBL-like protein kinase which was expressed higher in roots and showed
 362 increased expression under 200 mM NaCl treatment (Guo, Halfter, Ishitani, & Zhu,
 363 2001). In the blue down-regulation module (Fig. 4f), *SUS4* (*Cluster1314*) encodes
 364 sucrose synthase 4, which was involved in response to hypoxia stress (Bieniawska et
 365 al., 2007). Therefore, these newly discovered hub genes might play crucial roles in the
 366 high salt stress associated regulatory network of *Spartina*.

367

368 **Complex gene expression dynamics of transporters and associated protein**
 369 **kinases**

Genome-wide comparison analysis and co-expression network analysis showed that ion transporters play important roles in high salt tolerance of *Spartina* (Fig. 3 and Table S8). To further explore the function of ion transporters, we investigated the gene expression dynamics of sodium and potassium transporters under high salt stress. Gene expression levels of each ion transporter for sodium and potassium transport and related protein kinases are listed in Table S9. In general, under high salt stress, most of the sodium and potassium transporters showed gene expression variation under high salt stress.

Genes involved in SOS pathways including Na^+/H^+ antiporter *SaSOS1* (*Cluster1424*) and the Na^+-K^+ homeostasis modulator *SaSOS2* (*Cluster19661*) were significantly induced under high salt stress. HKT1 restricts long-distance transport of Na^+ from root to leave and therefore has antagonistic functions with SOS1 (J. K. Zhu, 2016). However, *SaHKT1* is not induced under high salt stress at the transcription level (Table S9). In *Spartina*, seven *SaAHAs* were identified and *SaAHA2* (*Cluster4145*) showed a significant up-regulation under high salt stress (Table S9). These results indicated that *SaAHA2* might be involved in the high salt tolerance of

386 *Spartina*.

387 SOS2 represents a large number of similar protein kinases, which include 10
388 SnRK2 and 25 SnRK3 in Arabidopsis (J. K. Zhu, 2016). In *Spartina*,
389 *SaSnRK2.10/SnRK2B* (Cluster27491) and *SaOST1/SnRK2.6/SRK2E* (Cluster17805)
390 from SnRK2 family are significantly up-regulated under high salt stress (Table S9).
391 However, *SaASK1/SnRK2.4/SRK2A* (Cluster27812) is not induced under high salt
392 stress (Table S9). Ten SnRK3s showed differential expression in salt stress by the
393 co-expression network analysis. Eight of them including *SaSnRK3.8/CIPK10*,
394 *SaSnRK3.9/CIPK12* (Cluster4217), *SaSnRK3.11/SOS2* (Cluster19661),
395 *SaSnRK3.14/CIPK6/SIP3* (Cluster23013), *SaSnRK3.16/CIPK1* (Cluster23029),
396 *SaSnRK3.22/CIPK11/SIP4* (Cluster25001), *SaSnRK3.24/CIPK5* (Cluster21691) and
397 *SaSnRK3.25/CIPK25* (Cluster23892) are significantly up-regulated under high salt
398 stress (Table S9). In addition, the expression of *SaSnRK3.12/CIPK9* (Cluster4692) is
399 increased under mild salt stress but decreased under high salt stress (Table S9).
400 However, *SaSnRK3.3/CIPK4* (Cluster28689) is the only down-regulated SnRK3s
401 under high salt stress (Table S9).

402 Ion transporters from Shaker family are responsible for potassium uptake and
 403 transport (Barragan et al., 2012; Lacombe et al., 2000). In *Spartina*, *SaAKT1* did not
 404 show any significant expression change under high salt stress, while *SaAKT2* showed
 405 a significant down-regulation (Table S9). The inward K⁺ channel *SaKAT2*
 406 (*Cluster24848*) was significantly up-regulated under high salt stress, while *SaKAT1*
 407 (*Cluster13882*) did not have significant change (Table S9). The K⁺ transport related
 408 *SaSKOR* (*Cluster12966*) gene was also up-regulated under high salt stress (Table S9).
 409 We also found that the expression of tonoplast localized K⁺/H⁺ antiporter *SaNHX2*
 410 (*Cluster17097*) was dramatically decreased at lower salt stress condition, while
 411 significant up-regulation was observed under high salt stress (Table S9).

412

413 **High salt stress induces global alternative splicing in *Spartina***

414 To investigate whether high salt stress affects AS in *Spartina*, 14,058
 415 non-redundant AS events corresponding to 20,431 transcripts were identified using
 416 our bioinformatics pipeline. Approximately 39% (5328/13,748) of unigenes with
 417 multiple isoforms are associated with AS events. Nearly half (2857/5328) of

AS-containing unigenes have more than two associated AS events (Fig. **5a**). The distribution of different types of AS in *Spartina* is comparable to that of other species (Reddy, Marquez, Kalyna, & Barta, 2013; Shen et al., 2014). Intron retention (IR) is the major AS type (56%). Most IR-AS events (93%) contain the canonical GT-AG splice site; the percentage of AS using alternative acceptor sites (AltA) is two times of that of alternative donor sites (AltD) (Fig. **5b**).

Importantly, 1154 AS events corresponding to 1627 transcripts are associated with salinity stress in *Spartina*, accounting for ~11% (578/5328) of AS-containing unigenes. The number of AS events is increased with growing salinity stress (Fig. **5c**), suggesting that high salt stress induces AS events in *Spartina*. In particular, IR remains the major AS type associated with salt stress (Fig. **5c**), and 233 IR events were identified in response to high salt stress (Table **S10**). According to the GO analysis, these AS events mainly affect photosynthesis-related unigenes (Fig. **S7**). Six IR events were selected for validation (Fig. **5d**). Levels of transcripts associated with these IR events were indeed increased under high salt stress when examined by both RT-PCR and qRT-PCR (Fig. **5e, f**). Overall, these results suggest that AS responds to

high salt stress in *Spartina*.

Functional validation of *Spartina* salt-tolerant unigenes in Arabidopsis

To test whether high salt stress responsive transcripts could be candidate gene resources for engineering salt-tolerant plants, we constitutively overexpressed heat shock protein coding gene- *SaHSP70-I* (*Cluster1653-042*) and NAC transcription factor- *SaAF2* (*Cluster30510-002*) in Arabidopsis. Two independent T₃ generation transgenic lines of overexpressed *SaHSP70-I* were grown at 120 and 150 mM NaCl. They showed better salt tolerance and had longer primary roots than control plants under salt stress (Fig. **5a-b**). Similarly, the salt tolerance analysis in Arabidopsis showed that plants with overexpressed *SaAF2* also exhibited increased salt tolerance (Fig. **5c-d**), indicating that *AF2* is a salt-tolerant candidate gene. The identification of many salt-responsive transcripts in *Spartina* and their functional validation in Arabidopsis further demonstrate the potential of using *Spartina* genes to engineer salt-tolerant plants.

450

451 **DISCUSSION**

452 **Overview of the *Spartina alterniflora* full-length transcriptome**

453 Salt stress is one of the most important environmental stresses in crop growth and
454 production. High salt stress severely delays crop growth and significantly reduces its
455 yield. *Spartina alterniflora* is a highly salt-tolerant monocotyledonous halophyte that
456 belongs to the *Poaceae* family. In this study, we performed comprehensive
457 transcriptome analysis in *Spartina* exposed to different salt stresses using the hybrids
458 of SMRT full-length transcriptome sequencing and RNA-seq. A total of 90,587
459 non-redundant full-length transcripts, 24,670 unigenes, 3494 ncRNAs, 1323 TFs and
460 1577 specific transcripts in *Spartina* were identified (Tables. **S3-6**). More importantly,
461 the sequence similarity analysis showed that approximately 80% of the full-length
462 transcripts in *Spartina* could be aligned to rice transcripts (Fig. **2d**), indicating that
463 *Spartina* is evolutionarily similar to rice. As *Spartina* is very similar to rice from
464 evolutionary aspects (Fig. **2d**), we also attempted to apply the *Spartina* unigenes for
465 engineering salt-tolerance plants. We over-expressed two *Spartina* salt

stress-responsive genes *SaHSP70-I* and *SaAF2* in Arabidopsis and observed that their transgenic lines confer salt-tolerance phenotypes (Fig. 6). Previous studies showed that the over-expression of *Spartina* vacuolar ATPase subunit c1 (*SaVHAc1*) and actin-depolymerizing factor (*SaADF2*) genes in rice promoted the salt tolerance of transgenic rice (Baisakh et al., 2012; Sengupta et al., 2019). Therefore, these results indicate that *Spartina* could be an ideal species for engineering salt tolerance in crops. Overall, the full-length transcriptome of *Spartina* potentially provides valuable resources for improving the salt tolerance of plants.

Gene expression dynamics of ion transporters under high salt stress

Genome-wide analysis showed that *Spartina* has a higher proportion of transporter related genes compared to rice and Arabidopsis (Fig. 3) and the co-expression network analysis also found that ion transporters are significantly enriched in the high salt stress associated module (Table S8). These results indicate that ion transporters may play a key role in the high salt tolerance of *Spartina*.

The most well known ion transporter under salt stress is SOS1 (Salt Overlay

482 Sensitive 1), a Na^+/H^+ antiporter that is used to extrude Na^+ from root cells into the
483 soil and load Na^+ into the xylem for long-distance transportation from roots to leaves
484 via transpiration systems (Yang & Guo, 2018; Y. Zhang et al., 2018; J. K. Zhu, 2002,
485 2016). Another important ion transporter is HKT1, which plays important roles in
486 long-distance Na^+ transport and has an antagonistic functions with SOS1 to restrict
487 long-distance transport of Na^+ from root to leave (J. K. Zhu, 2016). It is proposed that
488 plants can activate *SOS1* under mild salt stress to transfer Na^+ from root to leave,
489 while HKT1 is activated under high salt stress to limit the transport of Na^+ from roots
490 because Na^+ accumulation in leaves exceeds its storage capacity (J. K. Zhu, 2016).
491 However, in *Spartina*, we showed that the expression level of *SaSOS1* is significantly
492 higher under high salt stress, while no significant gene expression change was
493 observed for *SaHKT1* (Table S9). These results suggest that the long-distance
494 transport of Na^+ from root to leave may continue to be active under high salt stress in
495 *Spartina*. One of the explanations is that leaves of *Spartina* have salt glands (Nestler,
496 1977; Subudhi & Baisakh, 2011) and can secrete salt, so that high expression of
497 *SaHKT1* may not be required to shut down Na^+ transport compared to other plants

498 (Jaime-Perez et al., 2017; M. Zhang et al., 2018).

499 It has been reported that the Na^+/K^+ ratio is very important in plant salt tolerance,
500 and salt stress increases Na^+ but leads to loss of K^+ (G. Chen et al., 2015; Y. Zhang et
501 al., 2018; X. Zhu et al., 2018). In general, *Spartina* maintained a higher K^+
502 accumulation under high salt stress via the active gene expression dynamics of
503 *SaAKT2*, *SaKAT2*, *SaSKOR* and *SaNHX2*. It is suggested that *AKT1* is a potassium
504 transporter under lower K^+ conditions for uptake of K^+ , while *AKT2* is one K^+ efflux
505 carrier under higher K^+ conditions (Y. Zhang et al., 2018). In *Spartina*, the reduction
506 in expression of *SaAKT2* but no change of *SaAKT1* (Table S9) may limit the K^+ efflux
507 under high salt stress and the results supported the physiologic observation that
508 *Spartina* has a higher K^+ accumulation level (Hester et al., 2001; Subudhi & Baisakh,
509 2011). Moreover, the down-regulation of *SaAKT2* may also reduce the Na^+ uptake
510 root cells initially through the K^+ channel via *SaAKT2* (Salvador-Recatala, 2016).
511 *KAT2* and *KAT1* have been reported to form inward K^+ channels in the form of
512 heterodimers and *KAT2* can also form active homotrimeric channels in the plasma
513 membrane (Nieves-Cordones et al., 2014). We found *SaKAT2* rather than *SaKAT1* is

514 significantly up-regulated under high salt stress (Table S9), suggesting that *SaKAT2*

515 may play a role in K^+ uptake and maintain a higher K^+ level through its homotrimeric

516 channels in *Spartina* under high salt stress. SKOR is involved in delivering of K^+

517 from stelar cells of roots to xylem toward the shoot (Gaymard et al., 1998). *SaSKOR*

518 is also up-regulated under high salt stress (Table S9), indicating higher K^+ transport

519 from root to leaf under high salt stress. More importantly, the result is consistent with

520 the observation that the lower Na^+ / K^+ ratio is present in *Spartina* leaves (Bradley &

521 Morris, 1991; Smart & Barko, 1980). Higher Na^+/K^+ ratio is considered to be the

522 major physiological reason for high salt tolerance of *Spartina* (Subudhi & Baisakh,

523 2011). The vacuolar is the largest dynamics reservoir of K^+ in plant cells and the

524 tonoplast localized K^+/H^+ antiporter, NHX1 and NHX2, are reported to mediate the

525 K^+ uptake from cytosol to vacuolar (Barragan et al., 2012). We found that *SaNHX2*

526 (*Cluster17097*) showed significant up-regulation under high salt stress (Table S9),

527 indicating that *SaNHX2* may involve in the K^+ reservoir in *Spartina* (Hester et al.,

528 2001).

529 Overall, these results indicate that the reduction of Na^+ exclusion, the activation of

530 Na^+ long-distant transport to secret them through salt gland and the higher K^+
531 accumulation under high salt stress may be several important high salt tolerance
532 mechanisms in *Spartina*.

533

534 **Protein kinase plays hub roles in the salt stress regulatory network**

535 Protein phosphorylation plays important regulatory roles in plants in response to
536 environmental stresses at the post-translational level (Y. Ding et al., 2015). The
537 CBL-interacting protein kinase SOS2 involves in the SOS regulatory pathway to
538 phosphorylate and activate the Na^+/H^+ antiporter SOS1 (J. K. Zhu, 2016). In *Spartina*,
539 the expression level of *SaSOS2* (*Cluster19661*) is significantly increased under high
540 salt stress (Table S9), indicating that *SaSOS2* is involved in high salt tolerance of
541 *Spartina*.

542 Moreover, three SnRK2s and 16 SnRK3s were identified in *Spartina* (Table S9).
543 Among them, two of *SnRK2s* and ten of *SnRK3s* showed significantly gene
544 expression dynamics under high salt stress (Table S9). In particular, *SaOST1* from
545 *SnRK2s* and *SaCIPK10* from *SnRK3s* belong to the top ten hub genes in the high salt

546 stress associated up-regulation modules (Fig. 4e). The OST1 is triggered by ABA
547 signaling under stress to phosphorylate the ABREs-binding proteins and activate the
548 expression of stress responsive genes (Furhata et al., 2006). For example, the OST1
549 interacts and phosphorylates the ICE1 from C-repeat binding factors dependent
550 pathways under cold stress to stable their proteins (Y. Ding et al., 2015). However,
551 how OST1 involves in the high salt stress remains elusive. *SaOST1* is present in the
552 top ten hub genes under high salt stress (Fig. 4e), suggesting that OST1 may also play
553 crucial roles in the high salt tolerance of *Spartina* via post-translational regulation of
554 salt stress related genes. CIPKs from many species including rice, maize and wheat
555 have been reported to play important roles in salt tolerance and the overexpression of
556 *CIPKs* significant improved salt tolerance of transgenic plants (X. Chen et al., 2014;
557 Deng et al., 2013; Xiang, Huang, & Xiong, 2007). However, it is unexplored whether
558 CIPKs play regulatory roles in salt tolerance. In this study, we showed that *SaCIPK10*
559 is among the top ten hubs genes of high salt stress associated up-regulation modules
560 in *Spartina* (Fig. 4e). These results suggest that *SaCIPK10* may play regulatory roles
561 in high salt tolerance. Interestingly, three of the top ten hub genes in the high salt

stress associated down-regulation module are *leucine-rich repeat receptor kinases* (*LRRs*) (Fig. 4f), indicating that LRRs may also involve in the regulation of high salt tolerance.

In summary, over one fourth of the hub genes from high salt stress associated regulator network are protein kinases from SnRKs or LRRs families, indicating that kinases may play important regulatory roles in high salt tolerance of *Spartina*.

The transcriptional and post-transcriptional regulation of photosynthesis related genes in *Spartina*

Photosynthesis is impaired under salt stress and it has been reported that *Spartina* saturates photosynthesis at relatively low CO₂ level (Bedre et al., 2016). At the molecular level, we showed that photosynthesis genes, especially those associated with photosynthetic pigment content and the performance of photosystem II (Brzezowski, Richter, & Grimm, 2015; Chaves, Flexas, & Pinheiro, 2009), are also down-regulated under high salt stress (Fig. 4c), indicating that photosynthesis is regulated at transcriptional level.

AS is one post-transcriptional regulatory mechanism used by plants to respond to different environmental stresses (Deng & Cao, 2017; F. Ding et al., 2014; Feng et al., 2015). In this study, transcriptome-wide analysis indicated that high salt stress affects global AS (Fig. 5, Table S10). In particular, high salt stress induces a higher number of AS events along salinity stress gradients (Fig. 5c). In addition, the percentage of salt stress-associated AS genes (11%) is very close to that found in cotton and Arabidopsis (10%) (F. Ding et al., 2014; J. Zhang et al., 2014). Although salt stress increased IR events in several key salt-responsive genes in Arabidopsis (F. Ding et al., 2014; Feng et al., 2015), we did not observe this phenomenon in *Spartina* (Fig. S7). However, interestingly, photosynthesis-related genes are significantly enriched in the high salt stress associated AS, indicating extensive regulation of this process (Fig. S7). Overall, our results suggest that photosynthesis is highly regulated at both transcriptional and post-transcriptional level in *Spartina* under high salt stress.

In summary, our results provide a glimpse of the high salt tolerance mechanism of *Spartina alterniflora*. In addition, the full-length reference sequences, splice isoforms, transcription factors, ncRNAs and high salt stress associated hub genes in

594 *Spartina* identified in this study will provide useful information for future research on
595 halophytes and engineering salt tolerant plants.

596

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608

609

610 **Author contributions**

611 L.M. conceived the ideas. L.M., X.W., G.J., and C.L. designed experiments. T.W.,
612 W.W., and F.L. performed the experiments. X.W., W.Y., S.L., S.Z., T.W. and Q.L.
613 contributed to data analysis and L.M. wrote the manuscript.

614 **Figure legends**

615 **Fig. 1** Overview of experimental and bioinformatics procedures in this study.

616 **Fig. 2** Characterization of the full-length transcriptome of *Spartina alterniflora*. (a)

617 Distribution of reads of insert (ROI) lengths in each size-selected library. (b)

618 Distribution of unique transcript clusters supported by different numbers of isoforms.

619 (c) Comparison of transcripts identified from RNA-seq and PacBio data. Contigs

620 from *de novo* assembly of RNA-seq data (Bedre *et al.*, 2016) were mapped to PacBio

621 full-length transcripts (FLs), and vice versa. nContigs: the number of *de*

622 *novo*-assembled contigs from RNA-seq data; nQ: the number of contigs mapped to

623 PacBio FLs; pQ: the percentage of contigs mapped to FLs; nS: the number of FLs

624 mapped to contigs; pS: the percentage of FLs mapped to contigs. (d) Sequence

625 similarity analysis of *Spartina* FLNC transcripts compared to rice and Arabidopsis

626 using BLAST (e-value < 1e-5).

627 **Fig. 3** Comparison of gene ontology category percentage among *Spartina alterniflora*,
628 rice and Arabidopsis. Blast2GO results of annotated genes or unigenes from these
629 three species were mapped to categories in the second level of GO terms. The p-value
630 was calculated using Fisher's exact test and double stars represent p-value below 0.01,
631 while a single star represents p-value between 0.01 and 0.05.

632 **Fig. 4** Identification of salt stress related transcripts and regulatory hub genes. **(a)**
633 Number of differentially expressed (DE) genes in 24 hours under different NaCl
634 stress conditions compared to control conditions. FC: fold change; up-regulated: FDR
635 < 0.05 and $\log_2FC \geq 1$; down-regulated: FDR < 0.05 and $\log_2FC \leq -1$. **(b-c)** Gene
636 ontology enrichment of high salt stress associated unigenes. **(b)** GO results for
637 up-regulated unigenes; **(c)** GO results for down-regulated unigenes. **(d)** Plot of
638 relative expression values of 15 DE genes from qRT-PCR and RNA-seq experiments.
639 Each point denotes the $\log_2$ (fold change) of expression levels between the respective
640 treated condition and the control condition. The Pearson correlation is 0.713 and the
641 p-value is 3.908e-08. **(e)** Top ten hub genes in the turquoise module of salt stress

regulatory network. Hub genes were ranked according to the maximal clique centrality (MCC) scores with colors indicating MCC scores from high (red) to low (white). (f) Top ten hub genes in the blue module of the salt stress regulatory network.

Fig. 5 Alternative splicing (AS) and high salt stress-associated AS in *Spartina*. (a) Distribution of unique transcript clusters supported by different numbers of AS events. (b) Proportion of different types of AS in *Spartina*. (c) Number of differentially expressed AS (DEAS) events associated with salinity stress. (d) Schematic showing the strategy used to validate intron-retention (IR) AS events. Primers were designed to specifically detect the expression of IR-containing transcripts by qRT-PCR under high salt stress, and a pair of primers flanking the IR region was used to validate the AS events by RT-PCR. (e) Relative expression of IR-containing transcripts measured by qRT-PCR. (f) Expression of IR transcripts and normal transcripts (NT) by RT-PCR.

Fig. 6 Functional validation of salt-responsive transcripts. (a) The salt tolerance phenotype of *SaHSP70-I* transgenic lines in Arabidopsis: The T₃ generation seedlings

of *SaHSP70-I* overexpression lines were grown on half-strength Murashige and
Skoog medium after 10 days of salinity stress treatment. (b) Overexpression of
SaHSP70-I confers better salt tolerance in Arabidopsis transgenic line. The primary
root lengths ($n > 20$) were measured with ImageJ. (c) The salt tolerance phenotype of
the *SaAF2* transgenic lines in Arabidopsis. (d) Overexpression of *SaAF2* improved
salt tolerance of Arabidopsis transgenic line ($n > 20$).

SUPPORTING INFORMATION

Supplemental Figures

Fig. S1 Gene ontology analysis of unigenes in *Spartina alterniflora*.

Fig. S2 Clusters with multiple isoforms showed higher expression levels than those
with a single isoform.

Fig. S3 RT-PCR validation of randomly selected transcripts in *Spartina alterniflora*.

Fig. S4 Heatmap showing the Pearson correlation of unigene expression levels in
different samples and repeated experiments.

Fig. S5 Differentially expressed transcripts and unigenes in different conditions.

674 **Fig. S6** RT-PCR experimental validation of salt-responsive unigenes.

675 **Fig. S7** GO enrichment analysis using transcripts involved in differentially expressed
 676 alternative splicing events.

677

678 **Supplemental Tables**

679 **Table S1.** List of primers used in this study.

680 **Table S2.** Summary of PacBio single-molecule long-read sequencing data.

681 **Table S3.** List of non-coding RNAs in *Spartina alterniflora*.

682 **Table S4.** List of transcription factor coding transcripts in *Spartina alterniflora*.

683 **Table S5.** Summary of transcription factor families in *Spartina alterniflora*.

684 **Table S6.** List of *Spartina*-specific transcripts.

685 **Table S7.** List of salt stress-responsive unigenes in *Spartina alterniflora*.

686 **Table S8.** Selective modules with diverse patterns in salt gradient experiment

687 **Table S9.** List of ion transporters and related protein kinases

688 **Table S10.** List of high salt stress associated intron retention events.

689

690

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Fig. 1 Overview of experimental and bioinformatics procedures in this study.

Fig. 2 Characterization of the full-length transcriptome of *Spartina alterniflora*.

Fig. 3 Comparison of gene ontology category percentage among *Spartina alterniflora*, rice and Arabidopsis.

Fig. 4 Identification of salt stress related transcripts and regulatory hub genes.

Fig. 5 Alternative splicing (AS) and high salt stress-associated AS in *Spartina*.

Fig. 6 Functional validation of salt-responsive transcripts.