



HAL
open science

Expression of a Heat Shock Protein 70 from the Brown Alga *Ectocarpus* sp. Imparts Salinity Stress Tolerance in *Arabidopsis thaliana*

Pramod Rathor, Tudor Borza, Ramin Bahmani, Sophia Stone, Thierry Tonon, Svetlana Yurgel, Philippe Potin, Balakrishnan Prithiviraj

► To cite this version:

Pramod Rathor, Tudor Borza, Ramin Bahmani, Sophia Stone, Thierry Tonon, et al.. Expression of a Heat Shock Protein 70 from the Brown Alga *Ectocarpus* sp. Imparts Salinity Stress Tolerance in *Arabidopsis thaliana*. *Journal of Applied Phycology*, 2023, 35 (2), pp.803-819. 10.1007/s10811-022-02897-7 . hal-04296583

HAL Id: hal-04296583

<https://hal.science/hal-04296583v1>

Submitted on 24 Nov 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright

Expression of a Heat Shock Protein 70 from the Brown Alga *Ectocarpus* sp. Imparts Salinity Stress Tolerance in *Arabidopsis thaliana*

Pramod Rathor^a, Tudor Borza^a, Ramin Bahmani^a, Sophia Stone^b, Thierry Tonon^{c,d}, Svetlana Yurgel^e, Philippe Potin^d, and Balakrishnan Prithiviraj^{a*}

^a Marine Bio-Products Research Laboratory, Department of Plant, Food and Environmental Sciences, Faculty of Agriculture, Dalhousie University, Nova Scotia, NS B2N5E3, Canada; pramod.rathor@dal.ca (P.R.); Tudor.Borza@dal.ca (T.B.); rbahmani@dal.ca (R.B.); syurgel@dal.ca (S.Y.); bprithiviraj@dal.ca (B.P.)

^b Department of Biology, Dalhousie University, Halifax, NS B3H 4R2, Canada; s.stone@dal.ca

^c Centre for Novel Agricultural Products, Department of Biology, University of York, Heslington, York YO10 5DD, UK; thierry.tonon@york.ac.uk

^d Integrative Biology of Marine Models (LBI2M), Station Biologique de Roscoff (SBR), Sorbonne Université, CNRS, UMR 8227, 29680 Roscoff, France; philippe.potin@sb-roscoff.fr

^e USDA-ARS, Grain Legume Genetics and Physiology Research Unit, Prosser, WA 99350, USA; Svetlana.yurgel@usda.gov

***Corresponding author:**

Balakrishnan Prithiviraj; Marine Bio-Products Research Laboratory, Department of Plant, Food and Environmental Sciences, Faculty of Agriculture, Dalhousie University, Nova Scotia, NS B2N5E3, Canada

E-mail : bprithiviraj@dal.ca

Phone: +1-902-9865720

1 **Abstract**

2 The brown alga *Ectocarpus* sp. belongs to Phaeophyceae, a class of macroalgae in the phylum
3 Heterokonts, which is also known as the Stramenopiles lineage. *Ectocarpus* is a dominant
4 seaweed in temperate regions around the globe, abundant mostly in the intertidal zones, a habitat
5 with harsh environmental conditions resulting from tidal cycles. Analysis of previously
6 generated transcriptomic data of brown alga showed the up-regulation of the *Esi0379_0027*
7 gene, encoding a HSP70 protein, in response to various abiotic stresses. Bioinformatics study
8 demonstrated that the HSP70 protein is soluble, monomeric and well conserved compared to
9 other organisms. **Subcellular localization of EsHSP70 revealed that the protein is localized in**
10 **nucleus.** To explore the role of *HSP70* in stress tolerance, it was expressed in *Arabidopsis* under
11 constitutive and stress inducible promoters. Transgenic *Arabidopsis* plants generated using both
12 promoters exhibited higher tolerance to salinity stress compared to wild type plant. Transcript
13 analysis of various abiotic stress-responsive genes showed that genes participating in Na⁺ efflux
14 and sequestration, and in ABA-mediated stress tolerance were remarkably upregulated in the
15 *EsHSP70*-expressing transgenic *Arabidopsis*. Overall, our results showed that the expression of
16 the *EsHSP70* promotes **salt stress tolerance** by upregulating stress responsive genes in
17 *Arabidopsis*.

18 | **Keywords:** *Arabidopsis thaliana*, *Ectocarpus* sp., [Phaeophyceae](#), heat shock protein, salinity

19
20
21
22
23
24
25

26 **1. Introduction**

27 Plants being sessile organisms are incapable to avoid stressful or unfavorable growth conditions
28 by changing place. These organisms are constantly exposed to several environmental stresses
29 such as salinity, drought, high temperature, heavy metal and ultra-violet radiation. These
30 stressors negatively affect plant growth and development leading to significant loss in
31 productivity. High soil salinity is the predominant among various abiotic stresses which causes
32 severe crop loss globally. In addition, the effect of global climate change further exasperates the
33 effects of abiotic stresses on agricultural production (Lesk et al. 2016). The crop loss due to these
34 stressors account up to 50% loss in production (Lobell et al. 2011). High salinity inhibits plant
35 growth in multiple ways such as osmotic stress, ion accumulation and secondary stress. The
36 primary stresses, such as salinity, drought and high temperature are often interconnected and lead
37 to secondary stresses such as oxidative stress. At cellular level, these stresses result in
38 physiological dehydration, which further damages cellular machinery (Wang et al. 2003).
39 Increased soil osmotic potential due to presence of salt reduces the plants ability to uptake water
40 and nutrients (Hasegawa et al. 2000). Accumulation of Na^+ and Cl^- ions in significantly higher
41 concentration than those of outside in the soil helps to maintain turgor pressure but cells must
42 strictly regulate contents of these ions in each cellular compartment to avoid inactivation of
43 enzymes thus prevent metabolic dysfunction (Munns and Gilliam 2015). Based on their ability
44 to tolerate salt stress, plants are classified as halophytes and glycophytes (Flowers et al. 1977);
45 halophytes are highly tolerant to salinity stress. These plants have evolved unique structural
46 features such salt secretory glands in leaves that has the capacity to excrete excess salt that
47 ensures their survival under higher levels of soil salinity. However, most of the crop plants are
48 glycophytic and lacks such structures therefore doesn't exhibit higher level of salinity stress
49 tolerance (~~Hasegawa et al. 2000~~; Julkowska and Testerink 2015; [Hasegawa et al. 2000](#)).
50 Generally, halophytes have higher concentration of Na^+ and Cl^- ions in leaves than the outside
51 environment that helps to maintain higher osmotic potential in the cells and therefore higher
52 turgor pressure. These plants store Na^+ and Cl^- in vacuoles and uses compatible solutes and K^+
53 ions to equilibrate the osmotic differences in different compartments of cytoplasm (Shabala
54 2013). Accumulation of Na^+ and Cl^- ions in some leaves may be deleterious but at whole plant
55 level it might be beneficial as plant can store the excess Na^+ and Cl^- ions in those leaves and later
56 remove these ions via abscission (Munns and Tester 2008).

57 Heat shock proteins are classified on the basis of their molecular weight into five major groups
58 named as sHSP (small heat shock proteins), HSP60, HSP70, HSP90 and HSP100 (Wang et al.
59 2004). These proteins are present and conserved in almost all living organisms from prokaryotes
60 to eukaryotes (De Maio 1999). Heat shock proteins have a vital role in developmental processes
61 and response to abiotic stresses like salt (Zou et al. 2012), drought (Cho and Choi 2009) and
62 extremes of temperature (Lopez-Matas et al. 2004). Among several HSPs, HSP70 is a highly
63 conserved protein and has been extensively studied in bacteria, plants and animals. It is the most
64 abundant molecular chaperon, and is localized to various cellular compartments including
65 cytosol, nucleus, mitochondria and endoplasmic reticulum, protecting proteins by folding of
66 misfolded or unfolded proteins, disassembly of protein complex to translocate in another cellular
67 compartment and in disaggregation of complex aggregates (Saibil 2013). HSP70 contains two
68 main domains: a substrate binding domain and an ATPase domain (Kampinga and Craig 2010;
69 Mayer 2010; Zuiderweg et al. 2012). The activity of HSP70 depends on the interaction between
70 these two domains and other co-chaperons (Saibil 2013). Proteins which are not folded correctly
71 in their native state contains several binding sites usually at every 30 to 40 residues for HSP70
72 (Rüdiger et al. 1997). It is known that HSP70 binds to the 7-residue segment of a polypeptide
73 chain which is hydrophobic in nature (Clerico et al. 2015). Even transient binding of polypeptide
74 segment with HSP70 can prevent the misfolding and aggregation of proteins thus maintains
75 protein in native state (Young 2010). Once reached to right destination polypeptide regains the
76 native state in free solution by detaching from HSP70. If it fails to refold properly it will lead to
77 rebinding to HSP70. Therefore, the function of HSP70 in proper folding seems to be in
78 stabilization of unfolded proteins until it can refold correctly (Sharma et al. 2010). **HSP70**
79 **proteins have been identified in several plant species (Vierling 1991; Boston et al. 1996). In**
80 ***Arabidopsis* 18 genes encoding the *HSP70* family proteins have been discovered. Analysis of the**
81 **expression profile of these genes showed that members of *HSP70* family were expressed in**
82 **response to several environmental stimuli such as high temperature, cold, drought, salinity and**
83 **other chemical stresses (Lin et al. 2001; Sung et al. 2001). Several studies have been carried out**
84 **and reported that overexpression of *HSP70* improved drought, salinity and high temperature**
85 **stress tolerance in transgenic plants. Cho and Choi (2009), reported that constitutive**
86 **overexpression of nuclear localized *HSP70* from *Nicotiana tabacum* improved high temperature**
87 **and drought stress tolerance in transgenic tobacco, whereas the antisense plants were highly**

88 susceptible (Cho and Choi 2009). Similarly, constitutive expression of *HSP70* from cotton
89 improved drought stress tolerance in transgenic tobacco (Ni et al. 2021). Overexpression of
90 *HSP70* from chrysanthemum have shown to improve salinity, drought and high temperature
91 stress tolerance in transgenic *Arabidopsis* (Song et al. 2014). *Arabidopsis* plants transformed
92 using fungal *HSP70* from *Trichoderma* improved tolerance to heat, salinity, osmotic and
93 oxidative stress (Montero-Barrientos et al. 2010).

94 Seaweeds are an integral component of marine coastal ecosystems, where they form large
95 dense forests and provides habitat for many organisms (Khan et al. 2009). Brown alga are
96 multicellular organisms belonging to Stramenopiles lineage (also known as Heterokonts) (Cock
97 et al. 2012). This lineage is distantly related to Archaeplastida (including green plants and red
98 algae) and Opisthokonts (including animal and fungi) (Baldauf 2003; Brodie et al. 2017). Since
99 each of these lineages have evolved independently it is thought that organisms in each of these
100 lineages have evolved mechanisms necessary for the development of complex multicellularity.
101 These mechanisms are well studied in land plants and in animals but very little is known in
102 brown algae (Coelho et al. 2012). During the billions year of evolution brown algae have made
103 the transition from unicellularity to complex multicellularity and therefore acquired novel
104 features which are absent in other lineages (Cock et al. 2012; Brodie et al. 2017). Endosymbiotic
105 gene transfer led to the significant incorporation of foreign genes and enriched the nuclear
106 genome. Some of these genes were targeted to specific organelle through N-terminal transit
107 peptide and many of these evolved novel functions in these organisms (Brodie et al. 2017).
108 These novel features make it very interesting to explore and decipher the metabolisms and
109 cellular biology of these organisms. Moreover, genome sequencing demonstrated that more than
110 36% of genes are of unknown functions and doesn't match to any other existing organisms. This
111 indicates that these are novel genes or got considerably diverged from the homologue genes
112 present in other organisms (Cock et al. 2012). Some of the novel features include metabolism of
113 halogenated compounds, accumulation of complex polysaccharides and higher tolerance to biotic
114 and abiotic stresses (Charrier et al. 2008). Moreover, in brown algae the photosynthetically fixed
115 carbon is not stored as starch granules, instead it is stored as laminarin and mannitol (Craigie
116 1974; Davis et al. 2003). In contrast to land plants, these organisms produce complex
117 polysaccharides such as alginates and sulfated fucans (Kloareg and Quatrano 1988). Genome
118 editing tools have been routinely applied to land plants to understand their complex organization.

119 However, their application in other lineages for instance in Stramenopile is very challenging as
120 these are not easily amenable, lack of standardized protocols for genetic transformation,
121 mutation, promoter analysis and subcellular localization. The genome sequencing of *Ectocarpus*
122 provided some insights to brown algal biology, however, various biological aspects still remains
123 largely unknown. Transcriptomics study on brown algae showed that the expression level of
124 several genes was upregulated under different abiotic stresses conditions, along with elevated
125 levels of stress tolerance (Ritter et al. 2014). One of these genes was identified as *EsHSP70*
126 (*Esi0379_0027*). In the present work, to examine the role of *EsHSP70* in abiotic stress tolerance,
127 it was expressed in *A-rabidopsis thaliana*. Transgenic *Arabidopsis* plants expressing *EsHSP70*
128 showed higher tolerance to salt stress. Furthermore, the transcript level of various stress-
129 responsive genes was found to be significantly higher in *HSP70*-expressing transgenic plants
130 compared to the control (normal condition without salt) plants.

131

132 2. Materials and Methods

133 2.1. Brown alga growth condition and RNA isolation

134 The axenic culture of brown algal model *Ectocarpus* sp. (Dilwyn) Lyngbye unialgal strain
135 32 (accession CCAP 1310/4, origin San Juan de Marcona, Peru) was established in a 10 liters-L
136 plastic tank filled with filtered, autoclaved natural sea water containing 1% (v/v) of provasoli
137 Provasoli nutrient medium. Culture was maintained at 14 °C in the growth room with light cycle
138 of 14h light/10h dark, with light intensity of 40 $\mu\text{mol-photons m}^{-2}\text{s}^{-1}$. Compressed air passing
139 through a filter was used to continuously air bubble the nutrient medium. The culture was then
140 treated with 1450 mM NaCl for 6 h to induce salt stress condition. Following the exposure to
141 high salinity stress condition culture was collected using filtration, carefully dried on absorbent
142 paper and promptly flash frozen in liquid nitrogen. Total RNA isolation was performed using 0.1
143 g of tissue following the protocol described earlier with minor modifications (Apt et al. 1995).
144 The modifications to the protocol were made as previously stated (Le Bail et al. 2008). One
145 microgram of RNA was treated with Turbo DNase (Ambion Austin, USA) following to the
146 manufacturer's instruction. The DNase-treated RNA was converted to cDNA, using a
147 SuperScript IV Reverse Transcriptase (Life Technologies, France), following the manufacturer's
148 procedures.

149

150

151 **2.2. Bioinformatics analysis**

152 The protein transmembrane helices or domain were identified using HMMTOP v.2.0
153 (<http://www.enzim.hu/hmmtop/>) and TMHMM v.2.0 (<https://services.healthtech.dtu.dk/>)
154 prediction servers. To determine the subcellular localization of protein, the amino acid sequence
155 was analyzed using TargetP 2.0, Signal P and DeepLoc-1.0 (<https://services.healthtech.dtu.dk/>),
156 iPSORT (<http://ipsort.hgc.jp>), and WoLF PSORT (<https://wolfpsort.hgc.jp>) prediction servers.
157 Homology modelling for secondary structure was carried out using PSIPRED (Buchan and Jones
158 2019) (<http://bioinf.cs.ucl.ac.uk/psipred>). To obtain information about the tertiary structure and
159 the folding of EsHSP70, protein model was generated using SWISS-MODEL (Waterhouse et al.
160 2018). The best model (77.64 % sequence identity) was built using bovine heat shock cognate 71
161 kDa as a template (SMTL ID: 4fl9.1; structure solved by X-ray diffraction 1.90 Å). BlastP
162 search was performed using EsHSP70 as a query to find the protein sequence homology with
163 **different other taxa**. MUSCLE alignments (Edgar 2004) of some selected protein sequences were
164 done using MEGA X software (Kumar et al. 2018). The evolutionary history was inferred by
165 using the Maximum Likelihood method and JTT matrix-based model (Jones et al. 1992). The
166 evolutionary analysis, comprised of 49 sequences including EsHSP70, analyses were performed
167 using MEGA X software (Kumar et al. 2018).

168 **2.3. Plasmid construction and transformation of Arabidopsis**

169 To create the plant transformation constructs, pEarleyGate 100 (Earley et al. 2006) and a
170 promoterless vector pMCS:GW (Michniewicz et al. 2015) were obtained from ABRC
171 (Columbus, OH, USA). Promoter sequence for a stress inducible gene *RESPONSIVE TO*
172 *DESICCATION 29A* (*RD29A*) was amplified from the wild type (Col-0) *Arabidopsis*. Gateway
173 vector containing stress inducible promoter of *RD29A* gene, was generated as described earlier
174 (Rathor et al. 2021). The coding sequence of *EsHSP70* was amplified by RT-PCR using gene
175 specific primers flanked by attB sequences and cloned into pDONR221 by the BP Clonase™ II
176 Gateway®, following the manufacturer's protocol to generate entry clone (Gateway® Technology
177 with Clonase II, Invitrogen, ~~Ontario~~, Canada). The entry clone harboring full gene sequence was

178 then recombined into pEarleyGate 100 (35S) and pMCS:GW (*RD29A* promoter) by LR
179 Clonase™ II Gateway®, following the manufacturer's protocol (Gateway® Technology,
180 Invitrogen, ~~Ontario~~, Canada). The correctness of the *RD29A* promoter and *EsHSP70* sequences
181 was confirmed by PCR using the forward primer sequence from the *RD29A* promoter and
182 reverse primer sequence from the *EsHSP70*. The expression clone was then transformed into
183 *Agrobacterium* strain GV310 (pMB90) using the freeze-thaw method followed by *Arabidopsis*
184 (ecotype Col-0) transformation through the floral dipping protocol (Clough and Bent 1998).
185 Positive transformants were screened as stated earlier (Rathor et al. 2021). Expression of the
186 transgene was analyzed in these lines using quantitative real time polymerase chain reaction
187 (qRT-PCR) as described in later section. For further experiments two homozygous independent
188 transgenic lines named as *EsHSP-Ox1* and *EsHSP-Ox2* (for 35S promoter) and three
189 homozygous independent lines for stress inducible promoter (*EsHSP-A*, B, and C) were used.

190 **2.4. Plasmid construction for subcellular localization**

191 To generate the transformation vector, pEarleyGate 103 (C-GFP-HIS) (Earley et al. 2006) was
192 obtained from ABRC. Stop codon was removed from the coding sequence of *EsHSP70* and the
193 entry clone produced as described in previous section. The entry clone harboring full gene
194 sequence without stop codon was then recombined into pEarleyGate 103 (C-GFP-HIS) using LR
195 Clonase™ II Gateway® as mentioned in previous section.

196 **2.5. Analysis of salinity stress tolerance**

197 Seeds of the wild type and transgenic *Arabidopsis* lines were germinated and grown
198 vertically on half strength Murashige and Skoog (MS) solid media (Phytotech, USA) for 4 days.
199 Uniform 4-day-old seedlings were selected and transferred on 1/2 MS solid media plates
200 containing 100 mM NaCl. Plates were placed vertically for 7 days at 22 °C with 16 h light/8 h
201 dark cycle, with light intensity of 100 $\mu\text{mol}\cdot\text{photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Primary root length was marked on
202 the day of seedling transfer. After 7 days, plates were scanned using a scanner (Epson
203 Expression 10000 XL, Epson, ~~Ontario~~, Canada), and increase in root length and the lateral roots
204 number was measured using Image J software (NIH, ~~United States~~USA). The percent leaf
205 chlorosis was recorded visually on 7th day after transfer. The seedlings were harvested on the 9th
206 day to determine the fresh weight, subsequently oven-dried for 5 days at 70 °C for dry weight
207 measurement. All ~~the~~ experiments were conducted in triplicate and 30 plants per each treatment

208 were examined. To investigate if the phenotypes observed in seedlings were evident in plants,
209 seeds of wild type and of *EsHSP70*-overexpressing lines were stratified for 2 days at 4 °C and
210 were sown directly on Jiffy peat pellets (Jiffy, ~~NB,~~ Canada). 13-day-old plants grown on peat
211 pellets were completely saturated with water to equilibrate amount of water in all peat pellets
212 across the treatments. After three days, they were treated with NaCl at the final concentration of
213 the 100 mM. The peat pellets moisture were kept equally during the experiment by irrigation
214 once every three days. Plants were photographed after 3 weeks and their fresh and dry biomass
215 were measured. Three independent experiments with five replications were performed.

216 In order to understand the effect of salinity stress on membrane intactness an experiment
217 for electrolyte leakage was performed. Plants were grown and treated as described above.
218 Following the exposure to salinity stress rosette leaves were harvested from 3 plants in one
219 biological replicate (5 biological replicates) and transferred into vials comprising 20 ml of
220 deionized water. Electrolyte leakage was measured using SympHony SB70C (VWR, ~~ON,~~
221 Canada) conductivity meter as described by (Cao et al. 2007).

222 **2.6. Plant RNA isolation and quantitative RT-PCR (qRT-PCR)**

223 The expression of abiotic stress associated key marker genes was examined in *EsHSP-Ox1*
224 (*35S promoter*) and *EsHSP-A (RD29A promoter)* lines. Plants were grown and treated as
225 described above. Total RNA was isolated from leaves at 24 and 120 h after salt stress treatment
226 using GeneJET plant RNA purification kit (Thermoscientific, ~~ON,~~ Canada). After quantification
227 of the RNA using a NanoDrop spectrophotometer (Thermoscientific, ~~ON, Canada~~), 2 µg of total
228 RNA was treated with RQ1 RNase free DNase following the manufacturer's protocol
229 (Promega, ~~ON,~~ Canada) and used for cDNA synthesis using the RevetAID cDNA Synthesis kit
230 according to the manufacturer's instructions (Thermoscientific, ~~ON, Canada~~). qRT-PCR was
231 conducted on the StepOne plus Real-Time PCR system (Applied Biosystems, ~~Ontario,~~ Canada),
232 using iTaq SYBR green (Bio-RAD, ~~ON,~~ Canada). The relative expression was determined by
233 normalization to the Actin transcript abundance (as an internal control) following the $2^{-\Delta\Delta CT}$
234 method (Pfaffl 2001). Finally, transcript levels were expressed relative to the treatment with
235 lowest expression. The oligonucleotide primer sequences are given in Supplemental Table S1.

236 **2.7 Protoplast isolation and transfection**

237 | Four weeks old *Arabidopsis* wild-type (Col-0) plant was used for protoplast isolation and
238 | transfection as previously described (Yoo et al. 2007). The transformed protoplasts were
239 | incubated for 16-18h at room temperature before imaging using an LSM 710 LASER scanning
240 | confocal microscope (Carl Zeiss, Canada) following the protocol stated earlier (Wu et al. 2009).

241 | **2.8. Statistical analyses**

242 | Analysis of Variance (ANOVA) was accomplished for all the data followed by Tukey post
243 | hoc test at $P \leq 0.05$ using Minitab 19.0 software (Minitab LLC, [State College, Pennsylvania,](#)
244 | [USA](#)).

245 | **3. Results**

246 | **3.1. Prediction of transmembrane domain and intracellular localization of EsHSP70**

247 | The presence of the transmembrane domains in EsHSP70 (*Esi0379_0027*) was analyzed
248 | using the HMMTOP v.2.0 and TMHMM v.2.0 online prediction servers, however,
249 | transmembrane domains was not identified in this protein. SignalP, iPSORT and TargetP 2.0
250 | online servers were employed to analyse the subcellular localization of EsHSP70 protein.
251 | According to the results, the presence of a mitochondrial signal peptide, chloroplast, or thylakoid
252 | luminal transfer peptide was ruled out. In plants, it is known that endogenous HSP70 is present
253 | in different cellular compartments and it is likely that EsHSP70 in transgenic plants likely to be
254 | same. However, final destination in particular cellular compartment may occur by interaction
255 | with other proteins. Protein structure prediction was performed based on the PSIPRED method
256 | and exhibited the secondary structure of EsHSP70 protein with a MW of 71.9 kDa and an
257 | isoelectric point (pI) of 5.0 (Fig. 1 a). The EsHSP70 protein structure was further explored using
258 | the SWISS-MODEL server. The best model (77.64% sequence identity) was obtained using
259 | bovine Heat shock cognate 71 kDa as a template (SMTL ID: 4f19.1). This suggested that
260 | EsHSP70 is a well-structured protein. Information coming from all these databases indicated that
261 | EsHSP70 is a soluble, monomeric protein (Fig. 1 b-d).

262 |

263 |

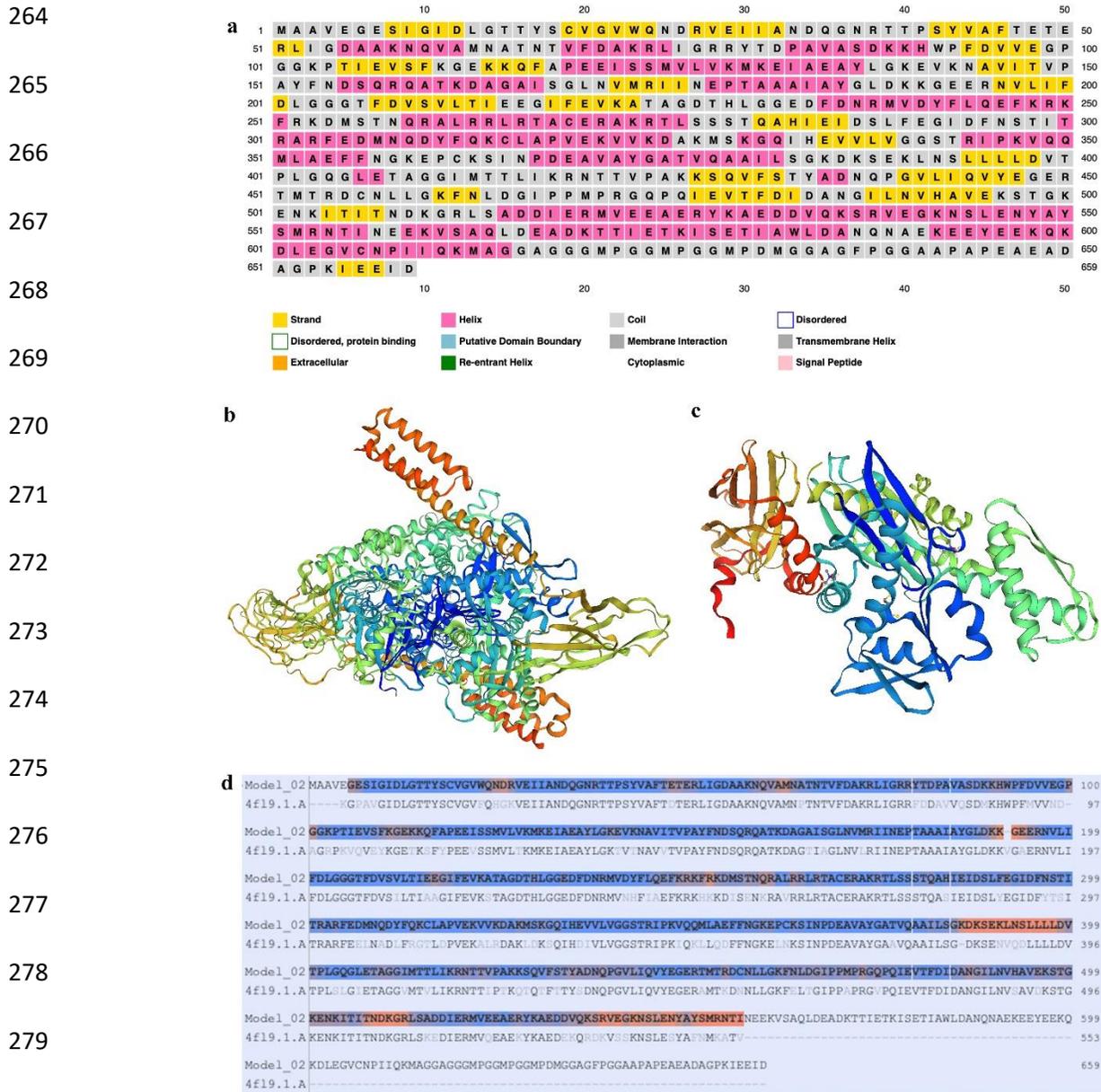
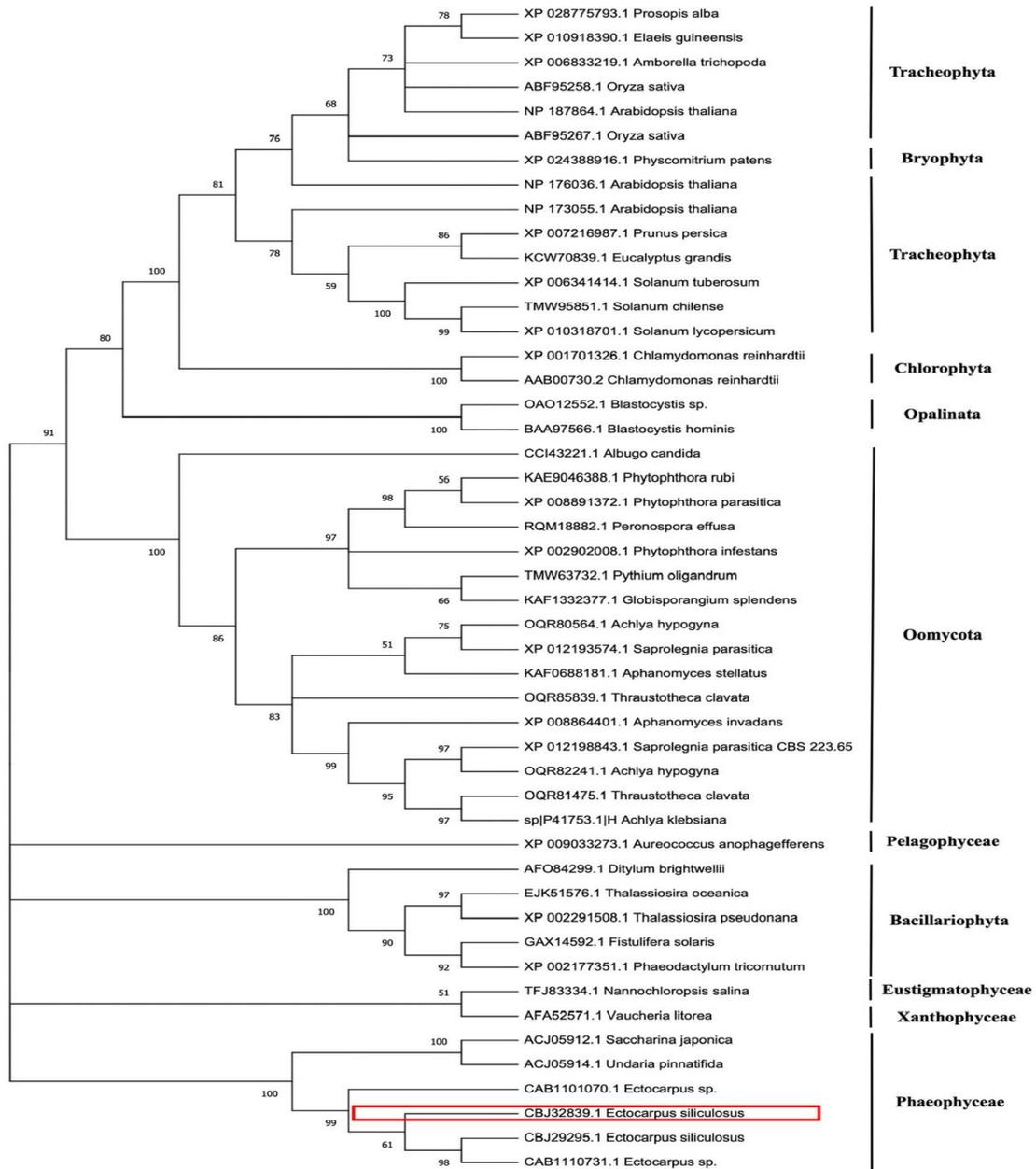


Fig 1. Structure prediction of EsHSP70 Protein.

(a) PsiPred sequence plot. The secondary structure prediction of EsHSP70. Model was generated at: <http://bioinf.cs.ucl.ac.uk/psipred/> (b) EsHSP70 three-dimensional (3D) structure modelling. 3D structure of the full length protein generated with ProMod3 3.0.0 in SWISS-MODEL. (c) Template (SMTL ID: 4f19.1). (d) Sequence alignment of EsHSP70 (Model_02) with bovine Heat shock cognate 71 kDa template.

287 **3.2. Phylogenetic analysis indicated that EsHSP70 considerable diverged from land plants**

288 Phylogenetic analyses of EsHSP70 (Fig. 2) showed the close evolutionary relationships
289 between *Ectocarpus* sp. sequences and other two brown alga (*Saccharina japonica* and *Undaria*
290 *pinnatifida*). Three sequences from *Ectocarpus* genus were in the same group as EsHSP70, while
291 these taxa are clustered separately from the Tracheophytes (vascular plants), and Bryophyta
292 (non-vascular land plants). These results implying that these *Ectocarpus* sp. sequences occurred
293 through duplication and divergent evolution.

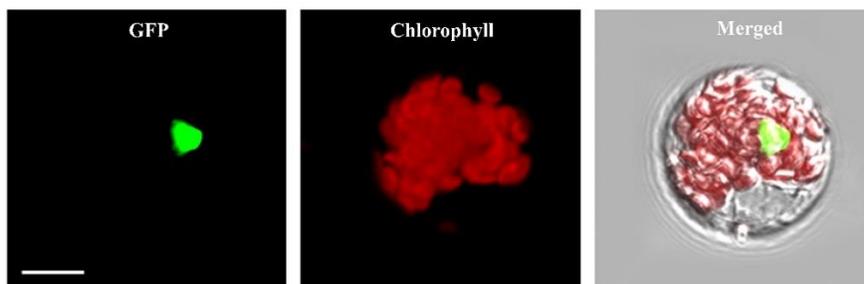


294

295 **Fig. 2.** Phylogenetic analysis of HSP70 in different species. The numbers of node indicate the
 296 percentages of bootstrapping after 500 replications. Phylogenetic tree was generated using
 297 Maximum Likelihood method and JTT matrix-based model. **Tracheophytes (vascular plants),**
 298 **Chlorophyta (green algae), Bryophyta (non-vascular land plants), Opalinata (non-phagotrophic**
 299 **heterokonts), Oomycote (fungus), Pelagophyceae (heterokont algae), Bacillariophyta (diatoms),**
 300 **Xanthophyceae (yellow-green algae), Eustigmatophytes–eustigmatophytes (eukaryotic algae),**
 301 **and Phaeophyceae (brown algae).**

302 **3.3. EsHSP70 is highly expressed in nucleus**

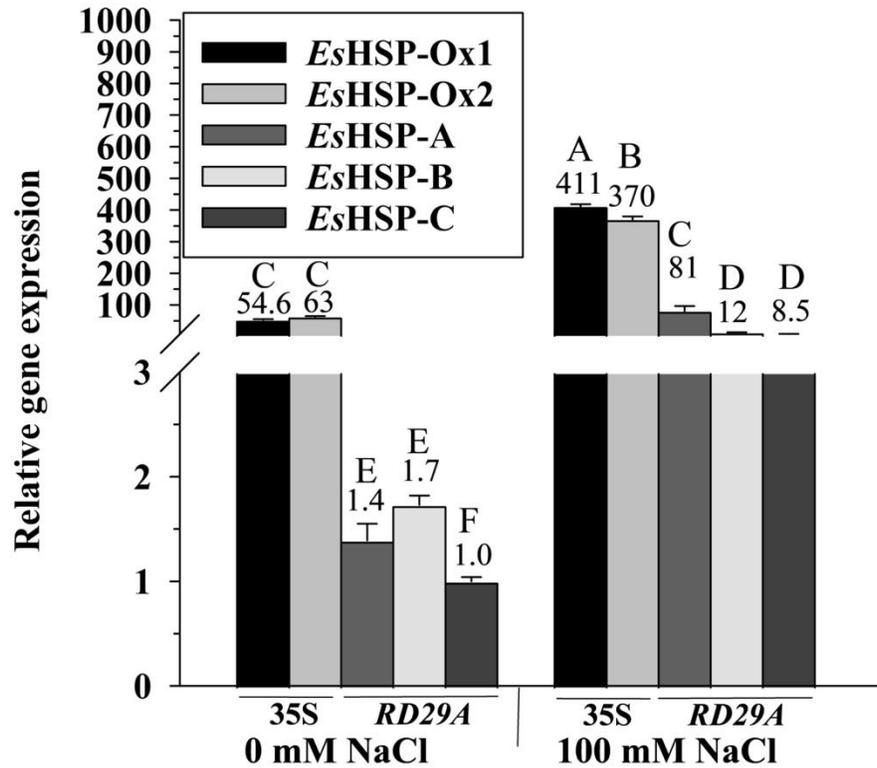
303 To determine the protein expression pattern, the C-terminal fusion construct of EsHSP70
304 with GFP tag was transiently expressed in *Arabidopsis* protoplast. Fluorescence microscopy
305 analysis of protoplast revealed the nucleus localization of the EsHSP70 (Fig. 3).
306



307
308 **Fig. 3.** Subcellular localization of EsHSP70. The coding region of EsHSP70 without stop codon
309 was fused to GFP under control of the constitutive 35S cauliflower mosaic virus promoter
310 (CaMV35S promoter). *Arabidopsis* protoplast was transformed with p35S::EsHSP70-GFP by
311 using PEG-mediated protocol. Scale bars = 20 μ m.

312 313 **3.4. Gene expression analysis shown that *EsHSP70* is abundantly expressed in *Arabidopsis*** 314 **under control and salt stress conditions**

315 To understand the role of this protein in abiotic stress tolerance it was expressed under 35S
316 and stress inducible promoter *RD29A*. Results of qPCR demonstrated that **in control condition**
317 **(normal condition without salt stress)**, the transcript level in the *EsHSP*-overexpressing lines
318 (*EsHSP*-Ox1 and -Ox2) was markedly high as compared to the *EsHSP*-A, *EsHSP*-B and *EsHSP*-
319 C lines (Fig.4). In *EsHSP*-Ox lines, having 35S promoter the expression level was very close to
320 as *actin*, the reference gene. Ct values for *actin* ranged between 21-22 cycles while those of
321 *EsHSP70* in *EsHSP*-Ox1 and *EsHSP*-Ox2 were 20 and 20.5, respectively, suggesting the
322 efficient transcription of *EsHSP70* and high mRNA stability in *A. thaliana*. When exposed to salt
323 stress, the gene expression level was remarkably enhanced in *EsHSP*-A, *EsHSP*-B and *EsHSP*-C
324 lines (57.8, 7.0 and 8.5 times, respectively), whereas in *EsHSP*-Ox1 and *EsHSP*-Ox2 the changes
325 in transcripts level were on the lower range compared to other transgenic lines (8.0 and 5.8
326 times, respectively). Further up-regulation of this gene in the 35S lines under salinity stress
327 suggests that mRNA is likely more stable under salinity stress (Shi et al. 2003).

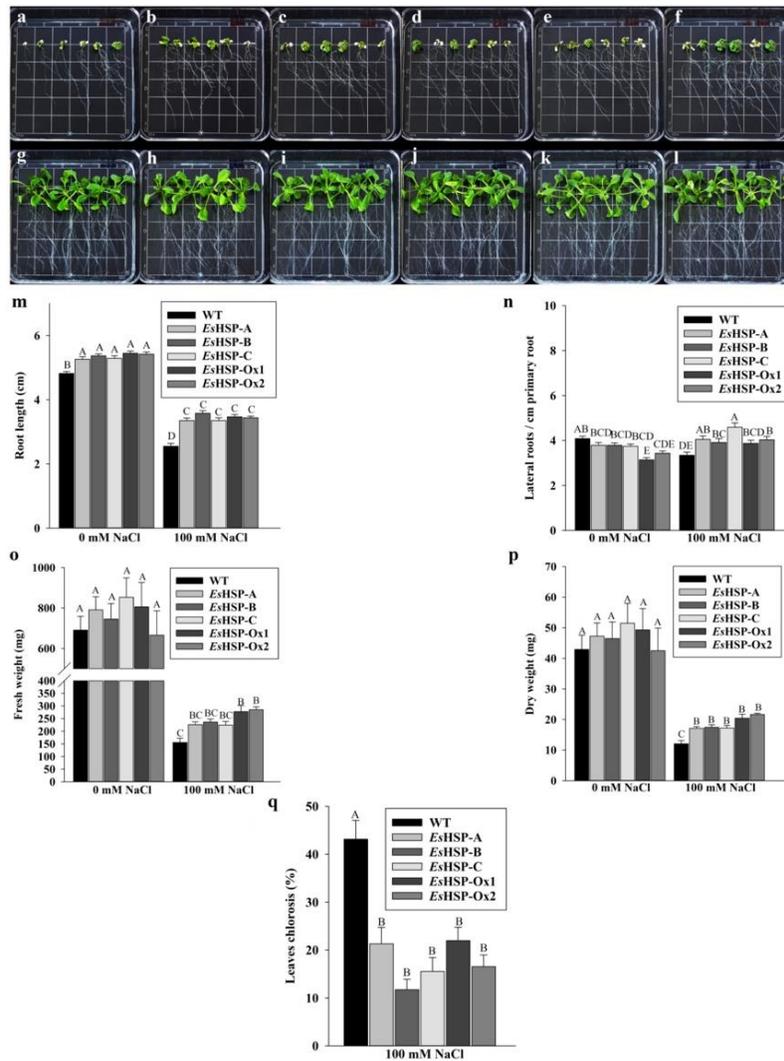


328

329 **Fig. 4.** Expression of *EsHSP70* in two independent lines under the 35S promoter and three
 330 independent lines harboring the stress inducible (*RD29A*) promoter under control (normal
 331 condition without salt stress) and salt stress conditions. The values on the bars indicate fold
 332 change ratio compared to the line with the lowest *EsHSP70* expression, *i.e.* *EsHSP-C* in normal
 333 condition. Different letters above the bars represent significant differences according to Tukey's
 334 test (p ≤ 0.05).

335 **3.5. Expression of *EsHSP70* ameliorated salt tolerance in *Arabidopsis***

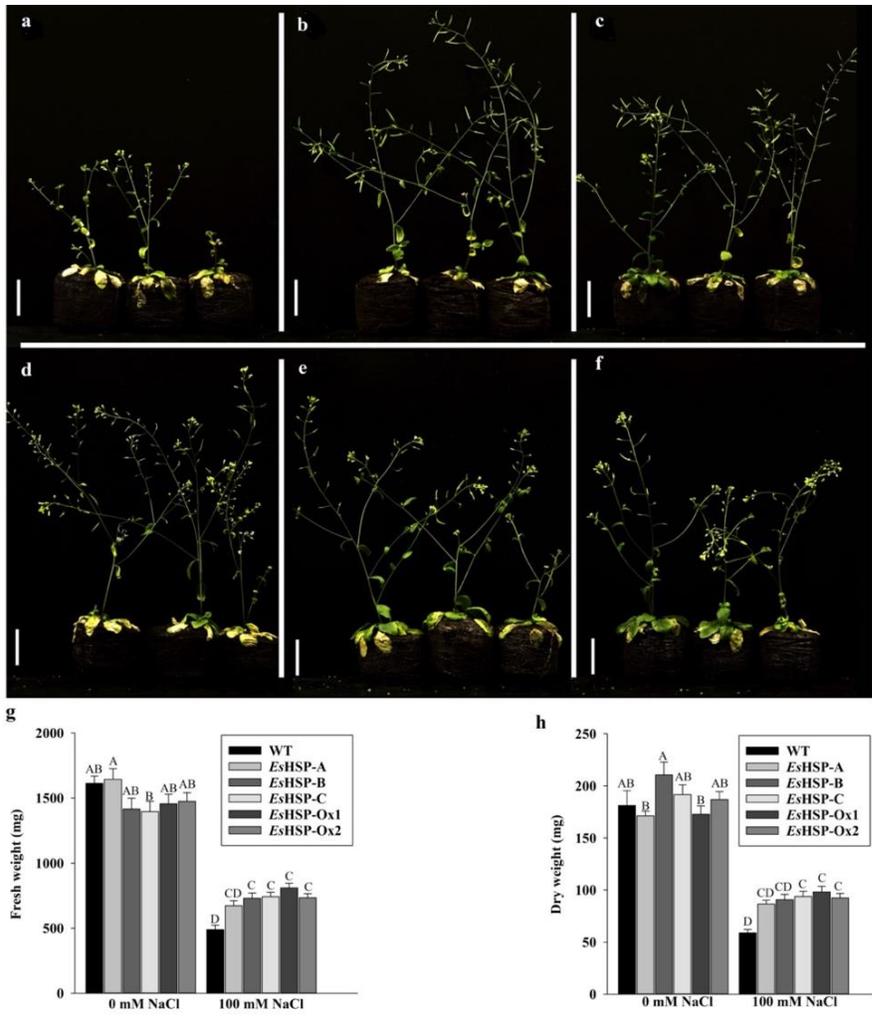
336 To investigate the salt stress tolerance of overexpression lines, 4-day-old seedlings grown
337 on 1/2 MS solid ~~media~~-medium plate were treated with 100 mM NaCl and further grown for 7
338 days. All the transgenic lines exhibited higher tolerance to salt stress (Fig. 5). After one week
339 following the exposure to salinity stress it was noticed that seedlings of overexpression lines had
340 significantly longer roots (12-13 and 9-10% increase under standard conditions and 35-36 and
341 30-40% increase under salinity stress for 35S and stress inducible overexpression lines,
342 respectively), higher number of lateral roots per cm of primary root (16-23 and 7-8% decrease
343 under standard conditions and 15-20 and 17-38% increase under salinity stress for 35S and stress
344 inducible overexpression lines, respectively), reduced leaf chlorosis (50-60 and 50-73% for 35S
345 and stress inducible overexpression lines, respectively), higher fresh weight (10-15 and 10-15%
346 increase under standard conditions and 75-80 and 45-50% increase under salinity stress for 35S
347 and stress inducible overexpression lines, respectively), and higher dry weight (14-15 and 10-
348 15% increase under standard conditions and 70-80 and 40-42% increase under salinity stress for
349 35S and stress inducible overexpression lines, respectively) in contrast to the wild type plants
350 (Fig. 5). Under *in-vivo* condition, wild type plants exposed to the salt stress, displayed severe
351 growth retardation in contrast to transgenic plants which continued to grow better (Fig. 6). In
352 response to salt stress, transgenic plants showed higher fresh weight (50-60 and 40-50% increase
353 under salinity stress for 35S and stress inducible overexpression lines, respectively) and dry
354 weight (60-65 and 45-60% increase under salinity stress for 35S and stress inducible
355 overexpression lines, respectively) in compared to the wild type plants (Fig. 6). Interestingly, no
356 significant differences were observed between transgenic lines generated using 35S promoter
357 and stress inducible promoter under both normal and salt stress condition (Fig. 7).



358

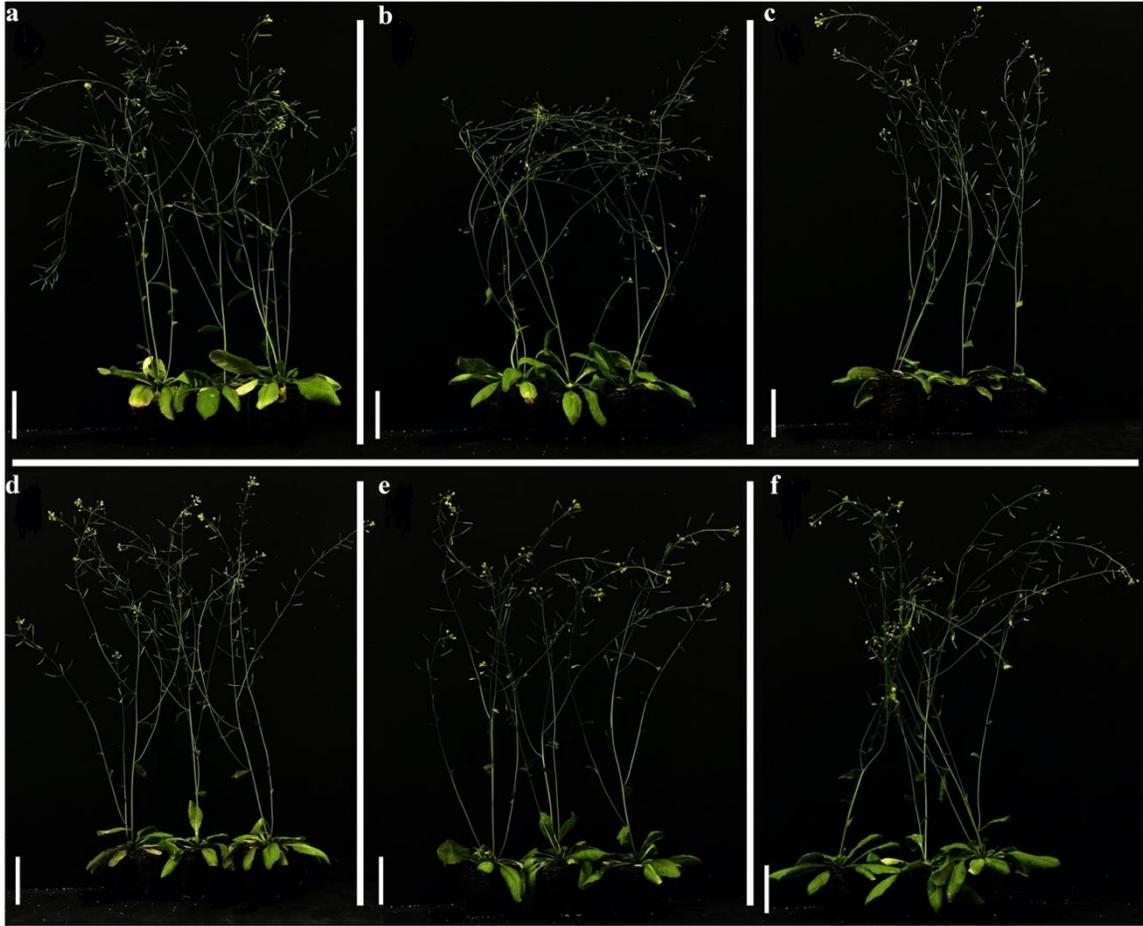
359 | **Fig. 5.** Salt stress tolerance in control and *EsHSP70*-expressing *Arabidopsis* under in-vitro
 360 | condition. (a, g) WT, (b, h) *EsHSP-A*, (c, i) *EsHSP-B*, (d, j) *EsHSP-C*, (e, k) *EsHSP-Ox1*, (f, l)
 361 | *EsHSP-Ox2*. Wild type, 2 independent 35S promoter (*EsHSP-Ox1-2*.) and 3 independent stress
 362 | inducible promoter (*EsHSPA-C*) transgenic *Arabidopsis* lines were treated with 0 (control) and
 363 | 100 mM NaCl. (a) to (f), seedlings grown under salt stress conditions (100mM NaCl). (g) to (l),
 364 | seedlings grown in normal conditions. Seedlings were photographed 9 days after transfer to the
 365 | control and 100 mM NaCl solid media. (m) root length, (n) number of lateral roots per cm of
 366 | primary root, (o) fresh weight, (p) dry weight and (q) leaf chlorosis of the wild type and
 367 | transgenic plant expressing *HSP70*. Values correspond to the means \pm SE (n = 3). Different
 368 | letters above the bars represent significant differences according to Tukey's test ($p \leq 0.05$).

369
 370
 371
 372
 373
 374
 375
 376
 377
 378
 379
 380
 381
 382



383 **Fig. 6.** Salt stress tolerance in wild type and *EsHSP70*-expressing *Arabidopsis* under in-vivo
 384 condition. Wild type, 2 independent 35S promoter (*EsHSP-Ox1-2*,) and 3 independent stress
 385 inducible promoter (*EsHSPA-C*) transgenic *Arabidopsis* lines were grown for 13 days under
 386 normal condition and subsequently treated with no (control) and 100 mM NaCl. (a) WT, (b)
 387 *EsHSP-A*, (c) *EsHSP-B*, (d) *EsHSP-C*, (e) *EsHSP-Ox1*, (f) *EsHSP-Ox2*, (g) fresh weight and (h)
 388 dry weight. The plants were photographed after 3 weeks. Values correspond to the means \pm SE
 389 (n = 3). Different letters above the bars represent significant differences according to Tukey's
 390 test ($p \leq 0.05$).

391



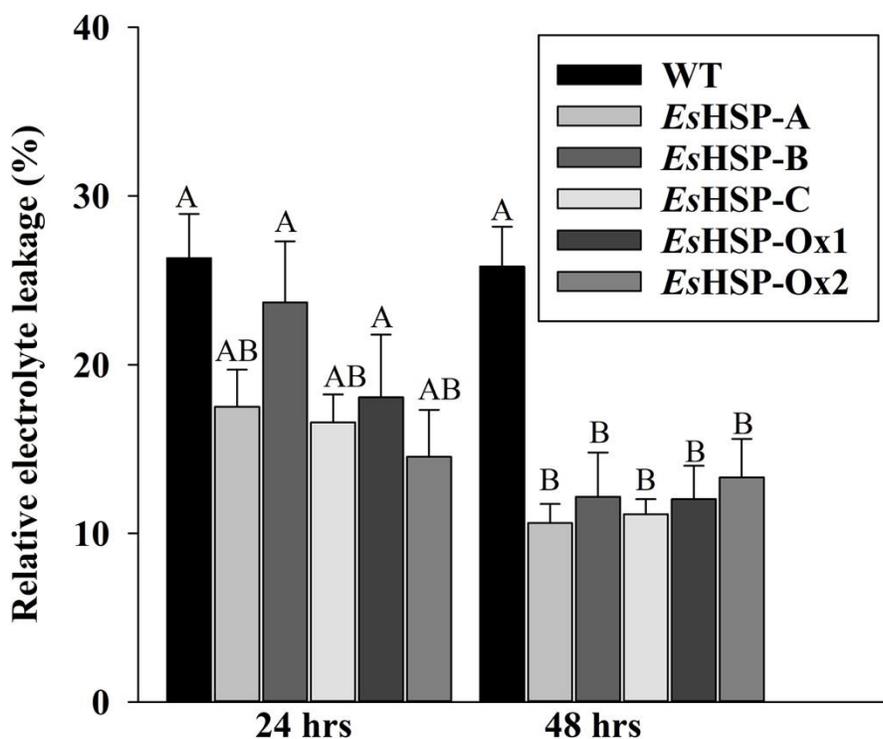
392

393 **Fig. 7.** Phenotype of wild type and 2 independent 35S promoter (*EsHSP-Ox1-2*,) and of 3
 394 independent stress inducible promoter (*EsHSPA-C*) transgenic *Arabidopsis* plants, expressing
 395 *EsHSP70*, in normal conditions. (a) WT, (b) *EsHSP-A*, (c) *EsHSP-B*, (d) *EsHSP-C*, (e) *EsHSP-*
 396 *Ox1*, (f) *EsHSP-Ox2*.

397 **3.6. Expression of *EsHSP70* demonstrated reduced leakage of electrolytes**

398 To examine the membrane stability under salt stress condition, 15-day-old wild type and
399 overexpression lines were exposed to 100 mM NaCl. Electrolyte leakage was estimated at 24 and
400 48 h after salinity stress exposure. Electrolyte leakage at 48 h was significantly lower in both 35S
401 and stress inducible promoter lines (50-53 and 52-60% in 35S and stress inducible
402 overexpression lines, respectively) in compared to control plants (wild type) suggesting that
403 membranes were highly intact in overexpression lines (Fig. 8).

404



405

406 **Fig. 8.** Electrolyte leakage in wild type and *EsHSP70*-expressing *Arabidopsis* under salt stress
407 condition. Wild type, 2 independent 35S promoter (*EsHSP-Ox1-2*) and of 3 independent stress
408 inducible promoter (*EsHSPA-C*) transgenic *Arabidopsis* plants were grown and treated as
409 described in materials and methods section. Values correspond to the means \pm SE (n = 5).
410 Different letters above the bars represent significant differences according to Tukey's test (p \leq
411 0.05).

412 3.7. Expression of the stress responsive genes is modulated in *EsHSP70*-expressing

413 *Arabidopsis*

414 To elucidate the mechanism by which *EsHSP70* improved salt tolerance in *Arabidopsis*,
415 the gene expression level of various stress responsive genes including *DREB2A* (*Dehydration-*
416 *Responsive Element-Binding Protein 2A*), *RD29A* (*Response to Desiccation 29A*), *RD29B*
417 (*Response to Desiccation 29B*), *RD26* (*Response to Desiccation 26*), *RD22* (*Response to*
418 *Desiccation 22*), *RD20* (*Response to Desiccation 20*), *RAB18* (*Responsive to ABA*), *LEA14* (*Late*
419 *Embryogenesis Abundant*), *HSP70* (*Heat Shock Protein 70*), *SOS1* (*Salt Overly Sensitive 1*) and
420 *NHX1* (*Sodium/Hydrogen Exchanger 1*) was measured in *EsHSP-Ox1*(35S promoter) and
421 *EsHSP-A* (*RD29A* promoter) by qRT-PCR. No significant change in transcript level of the
422 studied genes was observed between wild type and transgenic lines in normal condition except
423 for *RAB18* that was 3.9-fold up-regulated in *EsHSP-A* at 120 h. Contrary to this, *EsHSP-Ox1*
424 and *EsHSP-A* exhibited higher expression level of the stress responsive genes after 24 h and 120
425 h salt stress exposure (Figs. 9 and 10). The expression level of *DREB2A* and *HSP70* genes was
426 more than 2-fold higher in *EsHSP-Ox1* in compared to wild type under both time points of the
427 salt stress condition (Figs. 9a and i, respectively). The expression of these genes was
428 significantly up-regulated in *EsHSP-A* line at both time points under salinity stress, and the
429 difference was 1.7 and 1.4-fold for *DREB2A* and 1.7 and 2.4-fold for *HSP70* (Figs. 10a and i,
430 respectively). In general, the expression level of *RD29A*, *RD29B*, *RD26*, *RD22*, and *RD20* was
431 enhanced (> 2-fold) at 24 h and reduced at 120 h salt stress conditions (Figs. 9b-f, respectively).
432 However, the expression of *RD29A*, and *RD26* was significantly different from wild type at 120
433 h (Figs. 9b and d, respectively). The expression of these genes except *RD22* was up-regulated in
434 *EsHSP-A* line. The difference was 2.0 and 1.8-fold for *RD29A*, 2.0 and 2.1-fold for *RD29B*, 1.9
435 and 2.2-fold for *RD26* and 1.9 and 1.5-fold for *RD20* (Figs. 10b-f, respectively). The Expression
436 of *RD22* was significantly different at 24 h salt stress but not at 120 h in *EsHSP-OX1* whereas no
437 significant differences were found in *EsHSP-A* line at both time points (Figs. 9e and 10e). The
438 expression of *RAB18* (coding for a protein from the dehydrin family), and *LEA14* were also
439 increased by salt stress in both *EsHSP-Ox1* and *EsHSP-A*, at both time points, and this
440 difference was 1.9 and 3.0-fold for *RAB18* and 1.6 and 1.9 fold for *LEA14* (Figs. 9g and h
441 respectively) in *EsHSP-OX1*, whereas the difference was 1.5-fold at both time points for *RAB18*
442 and 1.5 and 1.3-fold for *LEA14* in *EsHSP-A* line (Figs. 10g and h respectively). Expression of

443 *SOS1*, an Na⁺/H⁺ antiporter, was significantly up-regulated in *EsHSP-Ox1* at both 24 h and 120 h
444 salt stress exposure by 1.5 and 2.4-fold, respectively (Fig. 9j), whereas no significant differences
445 were observed in *EsHSP-A* line at both time points (Fig. 10j). The expression of *NHX1*, an
446 Na⁺/H⁺ antiporter showed 1.5-fold induction in *EsHSP-OX1* and 1.3-fold induction in *EsHSP-A*
447 at 24 h and no significant difference was observed at 120 h in both lines (Figs. 9k and 10k).

448

449

450

451

452

453

454

455

456

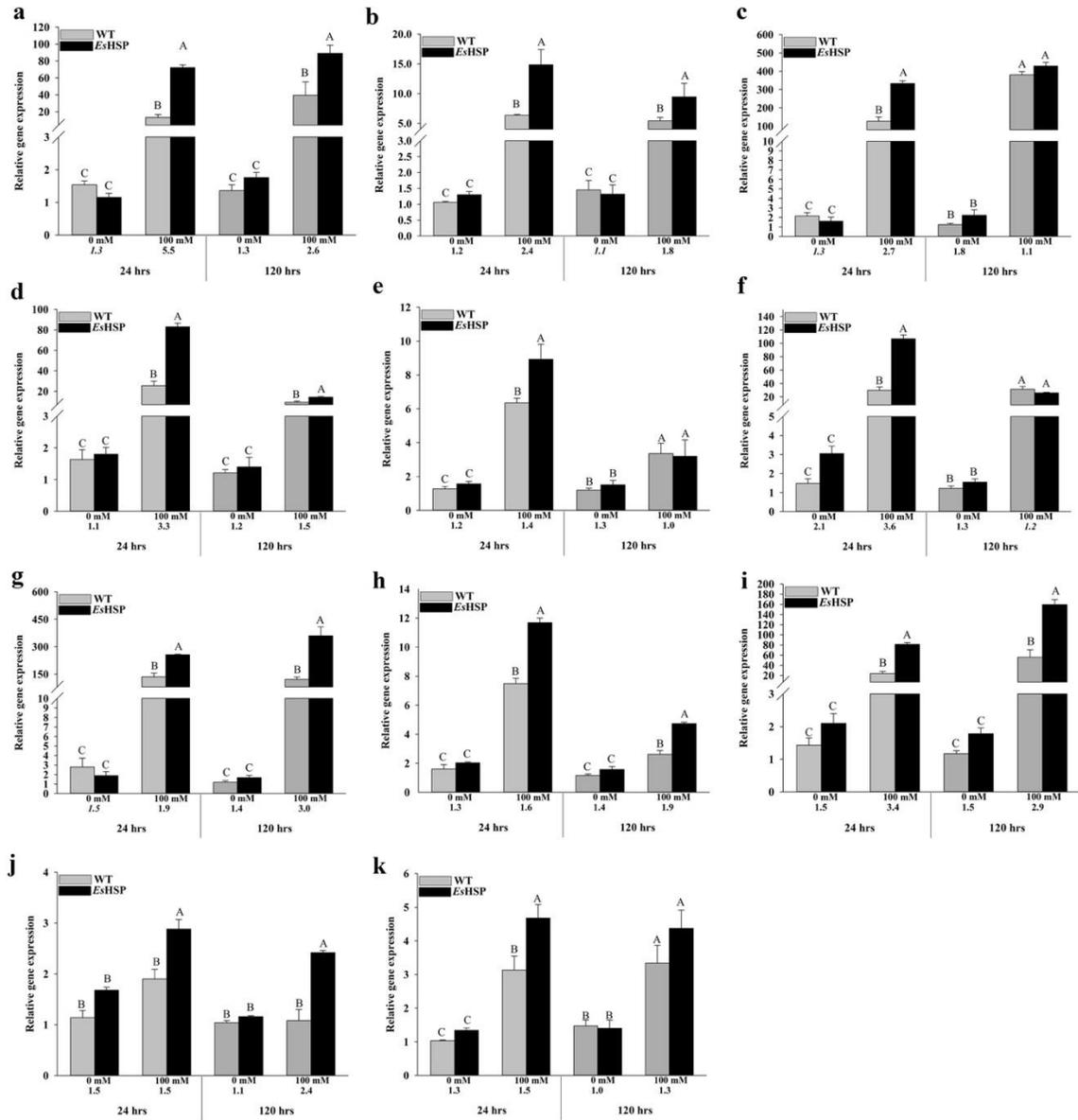
457

458

459

460

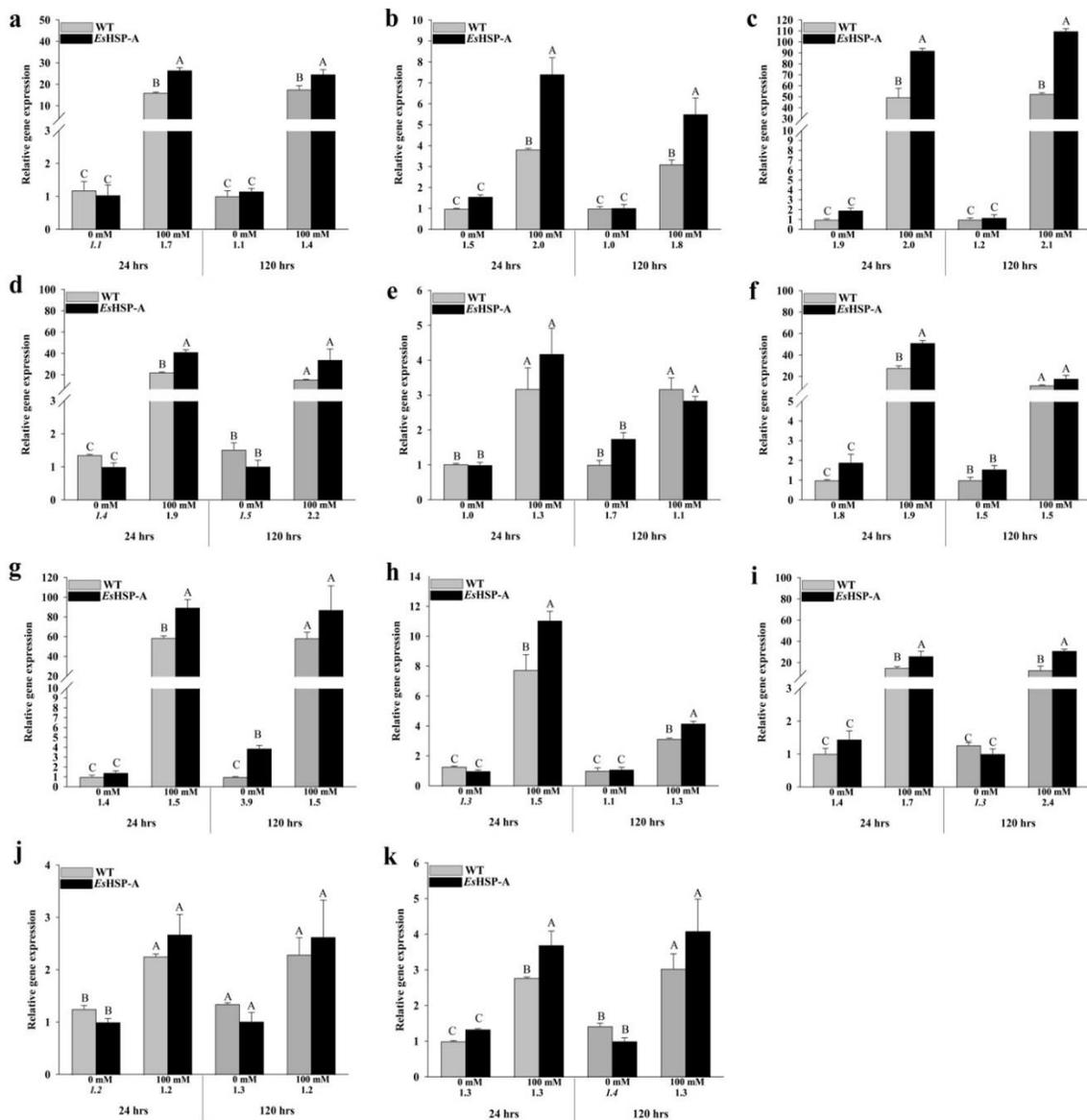
461



462

463 **Fig 9.** Expression of stress inducible genes in response to salt stress in *EsHSP-Ox1(35S*
 464 *promoter)* and wild type plants. qRT-PCR results indicating the transcript levels of various stress
 465 inducible genes in the wild type and *EsHSP70-Arabidopsis* under normal (control) and salt stress
 466 condition (100 mM NaCl for 24 h and 120 h). *Actin* was used as the internal control and
 467 transcript levels were normalized to the individual with the lowest expression. Values under the
 468 bars indicate WT fold difference. Default font values represent up-regulation while italicised values
 469 represent down-regulation. Data represents mean \pm SE (n=3). (a) *DREB2A*, (b) *RD29A*, (c)
 470 *RD29B*, (d) *RD26*, (e) *RD22*, (f) *RD20*, (g) *RAB 18*, (h) *LEA14*, (i) *HSP70*, (j) *SOS1* and (k)

471 *NHX1*. Different letters above the bars represent significant differences according to Tukey's test
 472 ($p \leq 0.05$).



473
 474 **Fig. 10.** Expression of stress inducible genes in response to salt stress in *EsHSP-A* (*RD29A*
 475 promoter) and wild type plants. qRT-PCR results indicating the transcript levels of various stress
 476 inducible genes in the wild type and *EsHSP70-Arabidopsis* under normal (control) and salt stress
 477 condition (100 mM NaCl for 24 h and 120 h). *Actin* was used as the internal control and
 478 transcript levels were normalized to the individual with the lowest expression. Values under the
 479 bars indicate fold difference. Default font values represent up-regulation while italicised values

480 represent down-regulation. Data represents mean \pm SE (n=3). (a) *DREB2A*, (b) *RD29A*, (c)
481 *RD29B*, (d) *RD26*, (e) *RD22*, (f) *RD20*, (g) *RAB 18*, (h) *LEA14*, (i) *HSP70*, (j) *SOS1* and (k)
482 *NHX1*. Different letters above the bars represent significant differences according to Tukey's test
483 ($p \leq 0.05$).

484 4. Discussion

485 Role of heat shock proteins have been extensively studied in bacteria, plants and animals.
486 Functions of HSP70 protein in response to abiotic stresses has been investigated in several crop
487 plants including rice, soybean, pepper, and *Arabidopsis* (Lin et al. 2001; Jung et al. 2013; Sarkar
488 et al. 2013; Zhang et al. 2015a; Zhang et al. 2015b). **Transcriptomics analysis of the brown alga**
489 ***Ectocarpus* sp. identified *HSP70* gene in this organism and similarity searches revealed that this**
490 **protein is well conserved.** This gene was consistently up-regulated in *Ectocarpus* sp. under
491 different abiotic stresses suggesting that it plays an important role to provide protection against
492 these physiological conditions in this organism (Ritter et al. 2014). To understand the function of
493 this gene we expressed it in *A. thaliana* under the 35S and stress inducible promoter
494 *RESPONSIVE TO DESSICATION 29A (RD29A)*. This is the first report on the overexpression of
495 *Ectocarpus* sp. *HSP70* improving high salinity stress tolerance in land plants.

496 Heat shock proteins, also known as chaperons, plays critical role in maintaining the metabolic
497 and structural integrity of cells by ensuring proper folding of misfolded or unfolded proteins and
498 preventing protein aggregation under abiotic stress conditions (Vierling 1991; Sung and Guy
499 2003; Wang et al. 2004). HSPs are shown to be induced by salinity stress in rice (Ngara and
500 Ndimba 2014), wheat (Sobhanian et al. 2011) and Poplar (Manaa et al. 2011). Previously,
501 transgenic plants have been generated to promote abiotic stress tolerance by expressing *HSP70*
502 gene from microbes and other land plants. *HSP70* from these two types of organism had higher
503 sequence similarity to the *HSP70* of other plants. For instance, overexpression of *HSP70* from
504 *Erianthus arundinaceus* has been shown to enhance salinity and drought stress tolerance of
505 sugarcane (Augustine et al. 2015). This increase in tolerance was due to higher membrane
506 stability and up-regulation of abiotic stress responsive genes including *DREB2A*, *LEA*, *RD29* and
507 *ERD*. It has been reported that overexpression of *HSP70* gene (*T34*) from *Trichoderma*
508 *harzianum* improved salinity stress tolerance in *Arabidopsis* (Montero-Barrientos et al. 2010).
509 Higher salt stress tolerance was ascribed to the higher expression level of *SOS1* and *APX1* in

510 transgenic plants. In another study, drought tolerance was enhanced by overexpression of
511 *NtHSP70-1* (Cho and Hong 2006). Similarly, *MtHSP70*-expressing transgenic *Arabidopsis*
512 showed higher drought tolerance (Li et al. 2017). Its multiple sequence alignment showed that it
513 had higher sequence similarity with other land plants including wheat, rice, corn and peas.
514 Transgenic *Arabidopsis* lines expressing chrysanthemum *HSP70* exhibited enhanced tolerance to
515 abiotic stress (Song et al. 2014). Taken together, all these studies suggest that *HSP70* plays an
516 important role in abiotic stress tolerance in plants. However, *HSP70* gene used in these studies
517 had high sequence similarity to the *HSP70* of the plants. In the current study, we isolated the
518 *HSP70* gene from brown algal model *Ectocarpus*. sp. Phylogenetic analysis indicated that this
519 gene got considerably diverged from *HSP70* in land plants as the close relatives were only two
520 brown algae and other algae from Xanthophyceae family. This gene has no similarity to *HSP70*
521 of the plants suggesting that this is a novel *HSP70*. Expression of *EsHSP70* in *A. thaliana*
522 significantly improved plant tolerance to salinity stress in all lines, irrespective of the promoter
523 used. It is worth mentioning that under both salinity stress and normal conditions, there was no
524 positive correlation between the *EsHSP70* transcript abundance and phenotypic data. Although,
525 *EsHSP70* expression was higher in 35S lines (constitutive over-expressed lines) than that of
526 RD29A lines (stress-inducible lines), they exhibited a similar phenotype (Figures 5, 7, and 8).
527 This might be due to the similar protein expression in these lines. Indeed, there is no correlation
528 between the transcript level and the final protein expression. It has been shown that several
529 processes such as translation rates, translation rate modulation, delay in protein synthesis, and
530 protein transport are significantly contribute in protein expression. This was further observed in
531 the transcriptomics and proteomics studies (Maier et al. 2009; Vogel and Marcotte 2012; Liu et
532 al. 2016). Furthermore, the elevated expression level of *EsHSP70* in constitutive over-expressed
533 lines, might already very high for the translational system leading to a saturation or a plateau
534 level.

535 Plants face alterations in their growing environment and responds to these variations by
536 adjusting their metabolism and physiology. The crucial changes during these alterations include
537 osmotic adjustment by production and accumulation of compatible solutes to maintain turgor
538 pressure, exclusion of Na⁺ ions, modulation of root architecture, shoot growth, organization of
539 leaves, leaf senescence, flowering time and biomass accumulation (Munns 2002; Sun et al. 2008;
540 Park et al. 2013). ~~Phytohormone~~ The phytohormone ABA plays a crucial role in plant tolerance

541 to abiotic stresses (Finkelstein et al. 2002). ABA is involved in the activation of the various
542 stress responsive genes, however, some of them are activated through an ABA-independent
543 pathway (Zhu 2002; Yamaguchi-Shinozaki and Shinozaki 2005). Stress responsive genes in
544 plant contain two main classes of cis acting elements referred as ABRE and DRE in the promoter
545 regions. ABRE functions in ABA dependent signaling pathway and DRE functions in ABA
546 independent pathway (Kim et al. 2011). In the present work, we analyzed the expression level of
547 various key marker genes associated to abiotic stress tolerance in *Arabidopsis* (i.e., *DREB2A*,
548 *RD29A*, *RD29B*, *RD26*, *RD22*, *RD20*, *RAB18*, *LEA14*, *HSP70*, *SOS1* and *NHX1*).

549 A DRE binding transcription factor *DREB2A* contains ethylene responsive factor elements
550 in promoter region (ERF/APETALA2). It functions as transcriptional activator of downstream
551 stress responsive genes by binding to DRE sequences present in promoter regions (Liu et al.
552 1998; Nakashima et al. 2000; Sakuma et al. 2002). The *DREB2A* expression was significantly
553 up-regulated in *EsHSP70*-expressing *Arabidopsis* under salt stress condition (at both 24 and 120
554 h) when compared to the ~~wild-wild~~-type plant (Figs. 9a and 10a). It was shown that the
555 overexpression of the *DREB2A* activated the expression of several downstream genes including
556 *RD29A*, *RD29B*, *RD17* and *LEA14* (Sakuma et al. 2006a). Likewise, the expression of these
557 genes i.e., *RD29A*, *RD29B* and *LEA14* was significantly up-regulated in *EsHSP70*-expressing
558 *Arabidopsis* when compared to the ~~wild-wild~~-type plant (Figs. 9 and 10b, c, and h, respectively).
559 Promoter region of these genes contain DRE suggesting that expression of these genes was
560 regulated by *DREB2A* (Sakuma et al. 2006b). Therefore, higher expression of these genes in
561 transgenic lines can be ascribed to the *EsHSP70*-mediated activation of the *DREB2A*. *RD26*
562 encodes NAC transcription factor functions in ABA dependent pathway as its expression was
563 up-regulated by salinity, drought, and ABA (Fujita et al. 2004). *RD20* encodes a Ca²⁺ binding
564 protein functions is directly regulated by *RD26* as the expression of this gene was up-regulated in
565 transgenic plants overexpressing *RD26* (Fujita et al. 2004). Our result showed the higher
566 expression of *RD26* and its target gene *RD20* in *EsHSP70*-expressing *Arabidopsis* line at both
567 time points for *RD26* and at 24 h for *RD20* (Figs. 9 and 10d and f, respectively).

568 Salinity stress causes disorganization of membrane and therefore results more electrolyte
569 leakage (Tuteja 2007). Electrolyte leakage is widely used to understand and measure the plant
570 tolerance to abiotic stresses. It determines the intactness of cellular membranes under abiotic

571 stress condition. In this study, a lower leakage of electrolytes was found in *EsHSP70*-expressing
572 plants under salt stress condition suggesting higher membrane stability in transgenic lines.
573 Several LEA proteins accumulated in response to abiotic stresses in plants. These proteins
574 protect macromolecules such as nucleic acid and enzymes by balancing redox and stabilization
575 of cellular membranes (Baker et al. 1988; Ingram and Bartels 1996; Veeranagamallaiah et al.
576 2011; Jia et al. 2014). Moreover, the expression level of LEA14 in *EsHSP70*-expressing
577 *Arabidopsis* was significantly higher than that of control (Figs. 9 and 10h). In support of our
578 result, Jia et al. (2014) demonstrated that overexpression of *LEA14* increased salt stress tolerance
579 (Jia et al. 2014).

580 The expression of *HSP70* was significantly up-regulated (> 2 fold) under salt stress
581 condition in [the](#) transgenic line in contrast to WT (Fig. 9 and 10i). Similarly, overexpression of
582 *HSP70* improved plant tolerance to high salinity, drought and temperature stress in *Arabidopsis*
583 (Wang et al. 2004) . To improve plant salt tolerance, it is important to prevent the entry of Na⁺
584 into plants to avoid its accumulation in the cytoplasm or organelles, except in the vacuoles. If
585 plant fails to restrict movement of Na⁺ from outside environment then it stores these toxic ion
586 into vacuoles by increased activity of transporters, and utilizes K⁺ and other compatible solutes
587 to balance the osmotic differences in cytoplasm thus reduces the concentration in cytoplasm to
588 prevent damage to cellular machinery (Shabala 2013). In *Arabidopsis*, *SOS1* has been shown
589 localized at plasma membrane (Shi et al. 2000; Qiu et al. 2002; Quintero et al. 2002; Shi et al.
590 2002). Based on its expression in different tissues it was suggested that it plays diverse role in
591 Na⁺ efflux from roots, reducing concentration of Na⁺ ions in cytoplasm (Zhu 2002).
592 Overexpression of *SOS1* increased tolerance to salt stress (Shi et al. 2003). *AtNHX1* encodes
593 Na⁺/H⁺ antiporter localized on tonoplast membrane of vacuoles. It functions in sequestration of
594 Na⁺ ions into vacuoles (Blumwald 2000). Overexpression of *NHX1* increased salt tolerance in
595 *Arabidopsis* (Gaxiola et al. 2001) and rice (Ohta et al. 2002). In our study, higher expression of
596 the *SOS1* and *NHX1* was observed in *EsHSP70*-expressing lines in compared to control under
597 salt stress condition (at 24 and 120 h) (Figs. 9 and 10j and k, respectively). Together, these
598 results suggest that overexpression of *EsHSP70* improves salinity stress tolerance by
599 transcriptional activation of *Arabidopsis* stress responsive genes.

600 **6. Conclusions**

601 In the present study, heterologous expression of the heat shock protein 70, from [the](#) brown alga
602 *Ectocarpus* sp., increased salt stress tolerance in *Arabidopsis*. According to the gene expression
603 analysis, higher tolerance of *HSP70*-expressing *Arabidopsis* can be ascribed to the higher
604 expression of the stress-responsive genes involved in ABA-induced abiotic stress tolerance, K⁺
605 homeostasis, and Na⁺ sequestration. Our results suggest that, in addition to the land plants and
606 microbes, brown algae can be employed as a genetic source to generate transgenic plants with
607 promoted tolerance to various abiotic stresses.

608 **Funding**

609 B.P.'s lab was supported by NSERC-DG grant 1177546, MITACS and France-Canada Research
610 Fund (FCRF). P.R. was supported by an NSERC Industrial Postgraduate Scholarship and a
611 MITACS Globalink Research Award. P.P. and T.T. were also supported by the Agence
612 Nationale de la Recherche via the investment expenditure program IDEALG Grant ANR-10-
613 BTBR-04.

614

615 **CRedit authorship contribution statement**

616 Pramod Rathor: Conceptualization, methodology, validation, formal analysis, investigation, data
617 curation, writing—original draft preparation, writing—review and editing, visualization. Tudor
618 Borza: Conceptualization, methodology, validation, formal analysis, investigation, data curation,
619 writing—original draft preparation, writing—review and editing, visualization. Ramin Bahmani:
620 formal analysis, investigation, validation, visualization, writing – review & editing. Sophia
621 Stone: writing – review & editing. Thierry Tonon: resources, writing—review and editing,
622 funding acquisition. Svetlana Yurgel: Writing – review & editing. Philippe Potin: resources,
623 writing—review and editing, funding acquisition. Balakrishnan Prithiviraj: Conceptualization,
624 methodology, supervision, project administration, writing—review and editing, funding
625 acquisition, resources.

626 **Data availability**

627 Data are available for the authors upon reasonable request.

628 **Declaration of competing interest**

629 The authors declare that they have no known competing financial interests or personal
630 relationships that could have appeared to influence the work reported in this paper.

631 **References**

- 632 Apt KE, Collier JL, Grossman AR (1995) Evolution of the
633 phycobiliproteins. *J Mol Biol* 248 (1):79-96
- 634 Augustine SM, Narayan JA, Syamaladevi DP, Appunu C, Chakravarthi M,
635 Ravichandran V, Subramonian N (2015) *Erianthus arundinaceus*
636 HSP70 (EaHSP70) overexpression increases drought and salinity
637 tolerance in sugarcane (*Saccharum* spp. hybrid). *Plant Sci* 232:23-
638 34
- 639 Baker J, Van dennSteele C, Dure L (1988) Sequence and characterization
640 of 6 Lea proteins and their genes from cotton. *Plant Mol Biol* 11
641 (3):277-291
- 642 Baldauf S (2003) The deep roots of eukaryotes. *Science* 300
643 (5626):1703-1706
- 644 Blumwald E (2000) Sodium transport and salt tolerance in plants. *Curr*
645 *Opin Cell Biol* 12 (4):431-434
- 646 Boston RS, Viitanen PV, Vierling E (1996) Molecular chaperones and
647 protein folding in plants. *Plant Mol Biol* 32 (1-2):191-222
- 648 Brodie J, Chan CX, De Clerck O, Cock JM, Coelho SM, Gachon C,
649 Grossman AR, Mock T, Raven JA, Smith AG (2017) The algal
650 revolution. *Trends Plant Sci* 22 (8):726-738
- 651 Buchan DW, Jones DT (2019) The PSIPRED protein analysis workbench:
652 20 years on. *Nucleic Acid Res* 47 (W1):W402-W407
- 653 Cao W-H, Liu J, He X-J, Mu R-L, Zhou H-L, Chen S-Y, Zhang J-S (2007)
654 Modulation of ethylene responses affects plant salt-stress
655 responses. *Plant Physiol* 143 (2):707-719
- 656 Charrier B, Coelho SM, Le Bail A, Tonon T, Michel G, Potin P, Kloareg B,
657 Boyen C, Peters AF, Cock JM (2008) Development and physiology
658 of the brown alga *Ectocarpus siliculosus*: two centuries of
659 research. *New Phytol* 177 (2):319-332
- 660 Cho EK, Choi YJ (2009) A nuclear-localized HSP70 confers
661 thermoprotective activity and drought-stress tolerance on plants.
662 *Biotechnol Lett* 31 (4):597-606

663 Cho EK, Hong CB (2006) Over-expression of tobacco NtHSP70-1
664 contributes to drought-stress tolerance in plants. *Plant Cell Rep* 25
665 (4):349-358

666 Clerico EM, Tilitsky JM, Meng W, Gierasch LM (2015) How *HSP70*
667 molecular machines interact with their substrates to mediate
668 diverse physiological functions. *J Mol Biol* 427 (7):1575-1588

669 Clough SJ, Bent AF (1998) Floral dip: a simplified method for
670 *Agrobacterium* - mediated transformation of *Arabidopsis*
671 *thaliana*. *Plant J* 16 (6):735-743

672 Cock JM, Sterck L, Ahmed S, Allen AE, Amoutzias G, Anthouard V,
673 Artiguenave F, Arun A, Aury J-M, Badger JH (2012) The *Ectocarpus*
674 genome and brown algal genomics: The *Ectocarpus* Genome
675 Consortium. In: ~~Adv. Bot. Res., vol 64: Elsevier, pp~~ 141-184

676 Coelho SM, Scornet D, Rousvoal S, Peters NT, Darteville L, Peters AF,
677 Cock JM (2012) *Ectocarpus*: A model organism for the brown
678 algae. *Cold Spring Harb* 2012 (2):pdb.emo065821

679 Craigie J (1974) Storage products. In: [Stewart WDP \(d\) Algal Physiol. Ogy](#)
680 [and Biochemistry., vol 10.](#) Blackwell Oxford, pp 206-237

681 Davis TA, Volesky B, Mucci A (2003) A review of the biochemistry of
682 heavy metal biosorption by brown algae. *Water Res* 37 (18):4311-
683 4330

684 De Maio A (1999) Heat shock proteins: facts, thoughts, and dreams.
685 *Shock* 11 (1):1-12

686 Earley KW, Haag JR, Pontes O, Opper K, Juehne T, Song K, Pikaard CS
687 (2006) Gateway - compatible vectors for plant functional
688 genomics and proteomics. *Plant J* 45 (4):616-629

689 Edgar RC (2004) MUSCLE: multiple sequence alignment with high
690 accuracy and high throughput. *Nucleic Acid Res* 32 (5):1792-1797

691 Finkelstein RR, Gampala SS, Rock CD (2002) Abscisic acid signaling in
692 seeds and seedlings. *Plant Cell* 14 (suppl 1):S15-S45

693 Flowers T, Troke P, Yeo A (1977) The mechanism of salt tolerance in
694 halophytes. *Annu Rev Plant Physiol* 28 (1):89-121

695 Fujita M, Fujita Y, Maruyama K, Seki M, Hiratsu K, Ohme - Takagi M,
696 Tran LSP, Yamaguchi - Shinozaki K, Shinozaki K (2004) A
697 dehydration - induced NAC protein, RD26, is involved in a novel
698 ABA - dependent stress - signaling pathway. *Plant J* 39 (6):863-
699 876

700 Gaxiola RA, Li J, Undurraga S, Dang LM, Allen GJ, Alper SL, Fink GR
701 (2001) Drought-and salt-tolerant plants result from
702 overexpression of the *AVP1 H⁺*-pump. *Proc Natl Acad Sci USA* 98
703 (20):11444-11449

704 Hasegawa PM, Bressan RA, Zhu J-K, Bohnert HJ (2000) Plant cellular and
705 molecular responses to high salinity. *Annu Rev Plant Biol* 51
706 (1):463-499

707 Ingram J, Bartels D (1996) The molecular basis of dehydration tolerance
708 in plants. *Annu Rev Plant Biol* 47 (1):377-403

709 Jia F, Qi S, Li H, Liu P, Li P, Wu C, Zheng C, Huang J (2014) Overexpression
710 of *Late Embryogenesis Abundant 14* enhances *Arabidopsis* salt
711 stress tolerance. *Biochem Bioph Res Co* 454 (4):505-511

712 Jones DT, Taylor WR, Thornton JM (1992) The rapid generation of
713 mutation data matrices from protein sequences. *Bioinformatics* 8
714 (3):275-282

715 Julkowska MM, Testerink C (2015) Tuning plant signaling and growth to
716 survive salt. *Trends Plant Sci* 20 (9):586-594

717 Jung K-H, Gho H-J, Nguyen MX, Kim S-R, An G (2013) Genome-wide
718 expression analysis of *HSP70* family genes in rice and identification
719 of a cytosolic *HSP70* gene highly induced under heat stress. *Funct*
720 *Integr Genomic* 13 (3):391-402

721 Kampinga HH, Craig EA (2010) The HSP70 chaperone machinery: J
722 proteins as drivers of functional specificity. [Nature reviews Rev](#)
723 [Molecular eCell biology Biol](#) 11 (8):579-592

724 Khan W, Rayirath UP, Subramanian S, Jithesh MN, Rayorath P, Hodges
725 DM, Critchley AT, Craigie JS, Norrie J, Prithviraj B (2009) Seaweed

726 extracts as biostimulants of plant growth and development. J
727 Plant Growth Regul 28 (4):386-399

728 Kim J-S, Mizoi J, Yoshida T, Fujita Y, Nakajima J, Ohori T, Todaka D,
729 Nakashima K, Hirayama T, Shinozaki K (2011) An ABRE promoter
730 sequence is involved in osmotic stress-responsive expression of
731 the *DREB2A* gene, which encodes a transcription factor regulating
732 drought-inducible genes in *Arabidopsis*. Plant Cell Physiol 52
733 (12):2136-2146

734 Kloareg B, Quatrano R (1988) Structure of the cell walls of marine algae
735 and ecophysiological functions of the matrix polysaccharides.
736 Oceanogr Mar Biol 26:259-315

737 Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular
738 evolutionary genetics analysis across computing platforms. Mol
739 Biol Evol 35 (6):1547-1549

740 Le Bail A, Dittami SM, De Franco P-O, Rousvoal S, Cock MJ, Tonon T,
741 Charrier B (2008) Normalisation genes for expression analyses in
742 the brown alga model *Ectocarpus siliculosus*. BMC Mol Biol 9
743 (1):75

744 Lesk C, Rowhani P, Ramankutty N (2016) Influence of extreme weather
745 disasters on global crop production. Nature 529 (7584):84-87

746 Li Z, Long R, Zhang T, Wang Z, Zhang F, Yang Q, Kang J, Sun Y (2017)
747 Molecular cloning and functional analysis of the drought tolerance
748 gene MsHSP70 from alfalfa (*Medicago sativa* L.). J Plant Res 130
749 (2):387-396

750 Lin BL, Wang JS, Liu HC, Chen RW, Meyer Y, Barakat A, Delseny M (2001)
751 Genomic analysis of the Hsp70 superfamily in *Arabidopsis*
752 *thaliana*. Cell Stress Chaperones 6 (3):201-208

753 Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K,
754 Shinozaki K (1998) Two transcription factors, DREB1 and DREB2,
755 with an EREBP/AP2 DNA binding domain separate two cellular
756 signal transduction pathways in drought-and low-temperature-
757 responsive gene expression, respectively, in *Arabidopsis*. Plant Cell
758 10 (8):1391-1406

- 759 Liu Y, Beyer A, Aebersold R (2016) On the dependency of cellular protein
760 levels on mRNA ~~Abundance~~[abundance](#). *Cell* 165 (3):535-550
- 761 Lobell DB, Schlenker W, Costa-Roberts J (2011) Climate trends and
762 global crop production since 1980. *Science* 333 (6042):616-620
- 763 Lopez-Matas MA, Nunez P, Soto A, Allona I, Casado R, Collada C,
764 Guevara MA, Aragoncillo C, Gomez L (2004) Protein cryoprotective
765 activity of a cytosolic small heat shock protein that accumulates
766 constitutively in chestnut stems and is up-regulated by low and
767 high temperatures. *Plant Physiol* 134 (4):1708-1717
- 768 Maier T, Guell M, Serrano L (2009) Correlation of mRNA and protein in
769 complex biological samples. *FEBS Lett* 583 (24):3966-3973
- 770 Manaa A, Ben Ahmed H, Valot B, Bouchet JP, Aschi-Smiti S, Causse M,
771 Faurobert M (2011) Salt and genotype impact on plant physiology
772 and root proteome variations in tomato. *J Exp Bot* 62 (8):2797-
773 2813
- 774 Mayer MP (2010) Gymnastics of molecular chaperones. ~~Molecular cell~~
775 [Cell](#) 39 (3):321-331
- 776 Michniewicz M, Frick EM, Strader LC (2015) Gateway-compatible tissue-
777 specific vectors for plant transformation. *BMC Res Notes* 8 (1):63
- 778 Montero-Barrientos M, Hermosa R, Cardoza RE, Gutierrez S, Nicolas C,
779 Monte E (2010) Transgenic expression of the *Trichoderma*
780 *harzianum* hsp70 gene increases *Arabidopsis* resistance to heat
781 and other abiotic stresses. *J Plant Physiol* 167 (8):659-665
- 782 Munns R (2002) Comparative physiology of salt and water stress. *Plant*
783 *Cell Environ* 25 (2):239-250
- 784 Munns R, Gilliam M (2015) Salinity tolerance of crops—what is the
785 cost? ~~New phytologist~~ [Phytol](#) 208 (3):668-673
- 786 Munns R, Tester M (2008) Mechanisms of salinity tolerance. *Annu Rev*
787 *Plant Biol* 59:651-681
- 788 Nakashima K, Shinwari ZK, Sakuma Y, Seki M, Miura S, Shinozaki K,
789 Yamaguchi-Shinozaki K (2000) Organization and expression of two
790 *Arabidopsis DREB2* genes encoding DRE-binding proteins involved

791 in dehydration-and high-salinity-responsive gene expression. *Plant*
792 *Mol Biol* 42 (4):657-665

793 Ngara R, Ndimba BK (2014) Understanding the complex nature of
794 salinity and drought-stress response in cereals using proteomics
795 technologies. *Proteomics* 14 (4-5):611-621

796 Ni Z, Liu N, Yu Y, Bi C, Chen Q, Qu Y (2021) The cotton 70-kDa heat shock
797 protein GhHSP70-26 plays a positive role in the drought stress
798 response. [Environmental and Experimental Botany](#) 191:104628

799 Ohta M, Hayashi Y, Nakashima A, Hamada A, Tanaka A, Nakamura T,
800 Hayakawa T (2002) Introduction of a Na⁺/H⁺ antiporter gene from
801 *Atriplex gmelini* confers salt tolerance to rice. [FEBS Letters](#) 532
802 (3):279-282

803 Park H, Lee S, You Y, Yoon D, Kim B-G, Ahn J, Cho H (2013) A rice
804 immunophilin gene, *OsFKBP16-3*, confers tolerance to
805 environmental stress in *Arabidopsis* and rice. *Int J Mol Sci* 14
806 (3):5899-5919

807 Pfaffl MW (2001) A new mathematical model for relative quantification
808 in real-time RT-PCR. *Nucleic Acids Res* 29 (9):e45

809 Qiu Q-S, Guo Y, Dietrich MA, Schumaker KS, Zhu J-K (2002) Regulation of
810 SOS1, a plasma membrane Na⁺/H⁺ exchanger in *Arabidopsis*
811 *thaliana*, by SOS2 and SOS3. *Proc Natl Acad Sci USA* 99 (12):8436-
812 8441

813 Quintero FJ, Ohta M, Shi H, Zhu J-K, Pardo JM (2002) Reconstitution in
814 yeast of the *Arabidopsis* SOS signaling pathway for Na⁺
815 homeostasis. [Proc Natl Acad Sci USA Proceedings of the National](#)
816 [Academy of Sciences](#) 99 (13):9061-9066

817 Rathor P, Borza T, Stone S, Tonon T, Yurgel S, Potin P, Prithviraj B (2021)
818 A novel protein from *Ectocarpus* sp. improves salinity and high
819 temperature stress tolerance in *Arabidopsis thaliana*. *Int J Mol Sci*
820 22 (4)

821 Ritter A, Dittami SM, Goulitquer S, Correa JA, Boyen C, Potin P, Tonon T
822 (2014) Transcriptomic and metabolomic analysis of copper stress

823 acclimation in *Ectocarpus siliculosus* highlights signaling and
824 tolerance mechanisms in brown algae. BMC Plant Biol 14 (1):116

825 Rüdiger S, Buchberger A, Bukau B (1997) Interaction of Hsp70
826 chaperones with substrates. Nature ~~structural biology~~[Struct Biol](#) 4
827 (5):342-349

828 Saibil H (2013) Chaperone machines for protein folding, unfolding and
829 disaggregation. Nat Rev Mol Cell Biol 14 (10):630-642

830 Sakuma Y, Liu Q, Dubouzet JG, Abe H, Shinozaki K, Yamaguchi-Shinozaki
831 K (2002) DNA-binding specificity of the ERF/AP2 domain of
832 *Arabidopsis* DREBs, transcription factors involved in dehydration-
833 and cold-inducible gene expression. Biochem Biophys Res
834 Commun 290 (3):998-1009

835 Sakuma Y, Maruyama K, Osakabe Y, Qin F, Seki M, Shinozaki K,
836 Yamaguchi-Shinozaki K (2006a) Functional analysis of an
837 *Arabidopsis* transcription factor, *DREB2A*, involved in drought-
838 responsive gene expression. Plant Cell 18 (5):1292-1309

839 Sakuma Y, Maruyama K, Qin F, Osakabe Y, Shinozaki K, Yamaguchi-
840 Shinozaki K (2006b) Dual function of an *Arabidopsis* transcription
841 factor *DREB2A* in water-stress-responsive and heat-stress-
842 responsive gene expression. Proc Natl Acad Sci USA 103
843 (49):18822-18827

844 Sarkar NK, Kundnani P, Grover A (2013) Functional analysis of Hsp70
845 superfamily proteins of rice (*Oryza sativa*). Cell ~~stress~~[Atress](#) and
846 Chaperones 18 (4):427-437

847 Shabala S (2013) Learning from halophytes: physiological basis and
848 strategies to improve abiotic stress tolerance in crops. ~~Annals of~~
849 ~~botany 112 (7):(Munns, 2015 #2573)(Munns, 2015 #2573)~~[Ann Bot](#)
850 [112](#):1209-1221

851 Sharma SK, De Los Rios P, Christen P, Lustig A, Goloubinoff P (2010) The
852 kinetic parameters and energy cost of the Hsp70 chaperone as a
853 polypeptide unfoldase. Nature ~~chemical biology~~[Chem Biol](#) 6
854 (12):914

- 855 | Shi H, Ishitani M, Kim C, Zhu J-K (2000) The *Arabidopsis thaliana* salt
856 | tolerance gene *SOS1* encodes a putative Na⁺/H⁺ antiporter.
857 | ~~Proceedings of the national academy of sciences~~ [Proc Natl Acad Sci](#)
858 | 97 (12):6896-6901
- 859 | Shi H, Lee B-h, Wu S-J, Zhu J-K (2003) Overexpression of a plasma
860 | membrane Na⁺/H⁺ antiporter gene improves salt tolerance in
861 | *Arabidopsis thaliana*. Nat Biotechnol 21 (1):81-85
- 862 | Shi H, Quintero FJ, Pardo JM, Zhu J-K (2002) The putative plasma
863 | membrane Na⁺/H⁺ antiporter *SOS1* controls long-distance Na⁺
864 | transport in plants. Plant Cell 14 (2):465-477
- 865 | Sobhanian H, Aghaei K, Komatsu S (2011) Changes in the plant
866 | proteome resulting from salt stress: toward the creation of salt-
867 | tolerant crops? J Proteomics 74 (8):1323-1337
- 868 | Song A, Zhu X, Chen F, Gao H, Jiang J, Chen S (2014) A chrysanthemum
869 | heat shock protein confers tolerance to abiotic stress. Int J Mol Sci
870 | 15 (3):5063-5078
- 871 | Sun F, Zhang W, Hu H, Li B, Wang Y, Zhao Y, Li K, Liu M, Li X (2008) Salt
872 | modulates gravity signaling pathway to regulate growth direction
873 | of primary roots in *Arabidopsis*. Plant Physiol 146 (1):178-188
- 874 | Sung DY, Guy CL (2003) Physiological and molecular assessment of
875 | altered expression of Hsc70-1 in *Arabidopsis*. Evidence for
876 | pleiotropic consequences. Plant Physiol 132 (2):979-987
- 877 | Sung DY, Vierling E, Guy CL (2001) Comprehensive expression profile
878 | analysis of the *Arabidopsis* Hsp70 gene family. Plant Physiol 126
879 | (2):789-800
- 880 | Tuteja N (2007) Mechanisms of high salinity tolerance in plants. ~~in:~~
881 | ~~Method Enzymol, vol 428, Elsevier, pp~~ 419-438
- 882 | Veeranagamallaiah G, Prasanthi J, Reddy KE, Pandurangaiah M, Babu
883 | OS, Sudhakar C (2011) Group 1 and 2 LEA protein expression
884 | correlates with a decrease in water stress induced protein
885 | aggregation in horsegram during germination and seedling
886 | growth. J Plant Physiol 168 (7):671-677

- 887 Vierling E (1991) The roles of heat shock proteins in plants. Annual Rev
888 Plant Biol 42 (1):579-620
- 889 Vogel C, Marcotte EM (2012) Insights into the regulation of protein
890 abundance from proteomic and transcriptomic analyses. Nature
891 Reviews Genetics 13 (4):227-232
- 892 Wang W, Vinocur B, Altman A (2003) Plant responses to drought,
893 salinity and extreme temperatures: towards genetic engineering
894 for stress tolerance. Planta 218 (1):1-14
- 895 Wang W, Vinocur B, Shoseyov O, Altman A (2004) Role of plant heat-
896 shock proteins and molecular chaperones in the abiotic stress
897 response. Trends Plant Sci 9 (5):244-252
- 898 Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R,
899 Heer FT, de Beer TAP, Rempfer C, Bordoli L (2018) SWISS-MODEL:
900 homology modelling of protein structures and complexes. Nucleic
901 Acid Res 46 (W1):W296-W303
- 902 Wu F-H, Shen S-C, Lee L-Y, Lee S-H, Chan M-T, Lin C-S (2009) Tape-
903 *Arabidopsis* Sandwich - a simpler *Arabidopsis* protoplast isolation
904 method. Plant Methods 5 (1):16
- 905 Yamaguchi-Shinozaki K, Shinozaki K (2005) Organization of cis-acting
906 regulatory elements in osmotic-and cold-stress-responsive
907 promoters. Trends Plant Sci 10 (2):88-94
- 908 Yoo S-D, Cho Y-H, Sheen J (2007) Arabidopsis mesophyll protoplasts: a
909 versatile cell system for transient gene expression analysis. Nature
910 Protocols 2 (7):1565-1572
- 911 Young JC (2010) Mechanisms of the Hsp70 chaperone system.
912 Biochemistry and Cell Biology 88 (2):291-300
- 913 Zhang J, Liu B, Li J, Zhang L, Wang Y, Zheng H, Lu M, Chen J (2015a) Hsf
914 and Hsp gene families in *Populus*: genome-wide identification,
915 organization and correlated expression during development and in
916 stress responses. BMC Genomics 16 (1):1-19
- 917 Zhang L, Zhao H-K, Dong Q-l, Zhang Y-y, Wang Y-m, Li H-y, Xing G-j, Li Q-
918 y, Dong Y-s (2015b) Genome-wide analysis and expression

919 profiling under heat and drought treatments of HSP70 gene family
920 in soybean (*Glycine max* L.). [Frontiers in Plant Science](#) 6:773
921 Zhu J-K (2002) Salt and drought stress signal transduction in plants. *Ann*
922 *Rev Plant Biol* 53 (1):247-273
923 Zou J, Liu C, Liu A, Zou D, Chen X (2012) Overexpression of OsHsp17.0
924 and OsHsp23.7 enhances drought and salt tolerance in rice. *J Plant*
925 *Physiol* 169 (6):628-635
926 Zuiderweg ER, Bertelsen EB, Rousaki A, Mayer MP, Gestwicki JE, Ahmad
927 A (2012) Allostery in the Hsp70 chaperone proteins. [In: Molecular](#)
928 [Chaperones. SpringerTop Curr Chem](#) 328: pp-99-153
929
930