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1-Aminocyclopropane-1-carboxylic acid and its analogs alleviate heat stress damage in the marine red alga *Neopyropia yezoensis* (Rhodophyta)

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Abstract
Heat stress disrupts algal growth, development, and physiological processes, such as photosynthesis, and eventually decreases seaweed productivity. Previous studies of crop plants have revealed that exogenous application of phytohormones prior or parallel to stress can alleviate the negative effects of abiotic stressors, including heat stress. However, there is limited information on phytohormone-induced tolerance to abiotic stressors in seaweed. In the present study, the application of the major plant hormones abscisic acid and salicylic acid failed to mitigate the negative effects of heat stress on the marine red alga *Neopyropia yezoensis*, whereas 1-aminocyclopropane-1-carboxylic acid (ACC), the direct precursor of the plant hormone ethylene, regulates thermotolerance. In addition, the ACC analogs 1-aminocyclobutane-1-carboxylic acid and α-aminoisobutyric acid enhanced tolerance to heat stress. ACC increased the expression of genes involved in antioxidant defense systems to protect photosynthesis and respiration. These results suggest ACC acts as a phytohormone to mitigate the impact on heat stress independent of ethylene in *N. yezoensis*.

Keywords *Neopyropia* · Red algae · Heat stress · 1-aminocyclopropane-1-carboxylic acid · Plant hormone

Introduction

Marine red algae in the order Bangiales, which includes the genera *Pyropia* and *Neopyropia* (formerly *Porphyra*), have been cultivated for several hundred years in Japan and are currently among the most successful aquaculture industries in East Asia, accounting for more than one billion US$ in revenue annually (Blouin et al. 2011). Bangiales contains high level of minerals (e.g., iron and zinc), vitamins (e.g., B12 and C), and proteins (Noda 1993), in addition to the sulfated polysaccharide porphyran, which has diverse physiological activities beneficial to human health, including antitumor, immunomodulating, anti-inflammatory, and antihyperlipidemic effects (Isaka et al. 2015). These benefits could substantially increase the market demand for Bangiales in the near future.

Abiotic stressors affect the growth, survival, cell division, photosynthesis, and subsequent quality and yield of Bangiales. Among potential abiotic stressors, heat stress can disrupt cellular homeostasis, leading to severe retardation of growth and development, and even death. Under heat stress conditions, the survival rate of conchospore germlings of *Neoporphyra haitanensis* was reduced to 15.9% after 15 days of culture at 28 °C (Yan et al. 2010). Thus, global warming is likely to significantly impact nori cultivation. Actually, during the early seeding period, nori farms are often exposed to sustained high temperatures followed by a drop to temperatures suitable for conchospore release, resulting in the inhibition of germling development, the induction of disease, and large-scale blade decay, resulting in a dramatic reduction in yield (Yan et al. 2010; Zhang et al. 2011).

Phytohormones are endogenous signaling molecules that play important roles in growth, development, and responses to various biotic and abiotic stressors, including heat stress (Verma et al. 2016; Li et al. 2021). The major plant hormones, including abscisic acid (ABA), cytokinins, salicylic acid (SA), jasmonic acid, ethylene, and brassinosteroids, play crucial roles in the response of land plants to heat stress (Verma et al. 2016; Li et al. 2021). Numerous studies have reported that exogenous applications of plant hormones prior or parallel to heat stress regulate thermotolerance of plants

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through the reactive oxygen species (ROS) signaling network (Khan et al. 2015; Devireddy et al. 2021; Kothari and Lachowiec 2021). In contrast to land plants, there have been relatively few reports of the effects of phytohormones on the thermotolerance of seaweed.

The non-proteinogenic amino acid 1-aminocyclopropane-1-carboxylic acid (ACC), the direct precursor of ethylene, is a simple two-carbon molecule with profound effects in higher plants (Lin et al. 2009; Van de Poel et al. 2015). Exogenous application of ACC has been used as a proxy for ethylene in numerous experiments over decades of research on ethylene signaling. However, recent findings have suggested that ACC also acts as a signaling molecule to regulate plant development and growth, independent of ethylene in the model plant *Arabidopsis thaliana* (Polko and Kieber 2019; Van de Poel 2020) and the basal land plant *Marchantia polymorpha* (Li et al. 2020; Katayose et al. 2021). Previous studies by our group showed that ACC stimulates the formation of sexual cells and protects gametophytes of *Pyropia*/*Neopyropia* species against oxidative stress (Uji et al. 2016; Yanagisawa et al. 2019). In addition, the exogenous ACC analog 1-aminocyclobutane-1-carboxylic acid (ACBC) induced gametogenesis and oxidative stress tolerance in the same manner as ACC, but not ethephon, an ethylene-releasing compound (Uji et al. 2020). Similarly, α-aminoisobutyric acid (AIB), a structural analog of ACC that blocks the conversion of ACC to ethylene in higher plants, mimics the effect of ACC to induce sexual reproduction without endogenous ACC accumulation (Endo et al. 2021). These findings suggest a possible role of ACC as a signaling molecule independent of ethylene in the regulation of sexual reproduction and stress tolerance in Bangiales.

An effective approach to improve heat tolerance in Bangiales is important for continued production in response to global warming. The objective of the present study was to assess the effect of exogenous plant hormones, including ACC, on conferring tolerance to heat stress in *Neopyropia yezoensis*, which is widely cultivated in Japan. The results revealed that the activation of ACC signaling can promote heat tolerance of *N. yezoensis* gametophytes by activation of genes associated with antioxidant defense systems by supporting photosynthesis and respiration.

Materials and methods

Algal material culture and pretreatment

The leafy gametophytes of *Neopyropia yezoensis* strain TU-1 were cultured in sterile vitamin-free Provasoli's enriched seawater (PES; Provasoli, 1968) as described previously (Uji and Mizuta 2021). Immature gametophytes (blade length, ~ 20 mm) were grown in a 90-mm diameter Petri dish with 40 mL of PES at 15 °C under a 10:14-h light:dark photoperiod using cool white fluorescent lamps at 40 µmol photons m^{-2} s^{-1}. Our previous study showed that 50 µM ACC was satisfactory to induce sexual reproduction in *N. yezoensis* (Uji et al. 2020), but application of 5 µM ACC weakly promoted it. In addition, a previous experiment revealed that ACC content in the gametophytes was in fairly small amounts (Endo et al. 2021). Based on these results, prior to heat stress treatment, the gametophytes were treated with 0, 5, 25, or 50 µM ACC (Tokyo Chemical Industry, Tokyo, Japan), 50 µM SA (Fujifilm Wako Pure Chemical Corporation, Japan), or 50 µM ABA (Tokyo Chemical Industry). To assess the effects of the ACC analogs, the gametophytes were also treated with 5 or 50 µM ACBC (Tokyo Chemical Industry) or AIB (Tokyo Chemical Industry). SA and ABA were dissolved in dimethyl sulfoxide (DMSO) to create stock solutions of 100 mM. ACC, ACBC, and AIB were dissolved in PES medium. Control experiments were performed with DMSO at concentrations corresponding to the maximum volume of the reagents.

Evaluation of tolerance to heat stress

To examine the effects of plant hormones on tolerance to heat stress, gametophytes were treated with chemical reagents for 7 days in a chamber at 15 °C under a 10:14-h light:dark photoperiod at 40 µmol photons m^{-2} s^{-1} and then transferred into a Petri dish containing new PES medium without chemical reagents and exposed to heat stress. The gametophytes were cultured for 2 weeks in a chamber at 28 °C under a 10:14-h light:dark photoperiod at 40 µmol photons m^{-2} s^{-1}. After the stress treatments, thalli were used for measurement of the maximum photochemical efficiency of photosystem II (PS II) (F_v/F_m) to evaluate heat stress-induced damage using a portable chlorophyll fluorometer (Opti-Science, Inc., Hudson, NH, USA.). Data are expressed as the mean ± standard deviation (SD) of five thalli for each condition.

Transcriptional analysis

Vegetative gametophytes (blade length, ~ 20 mm; 0.1-g fresh weight) were cultured in 100 mL of medium containing 50 µM ACC for 0, 3, or 7 days, then were frozen with liquid nitrogen and stored at − 80 °C until RNA extraction. RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR) analysis were performed as described by Uji et al. (2019). Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen, Germany) in liquid nitrogen with a mortar and pestle, in accordance with the manufacturer's instructions. The extracted RNA was purified with the TURBO DNA-free kit (Invitrogen/Life Technologies, USA) to obtain DNA-free RNA. First-strand cDNA was

synthesized from 0.5 µg of total RNA using the PrimeScript II 1st strand cDNA Synthesis Kit (TaKaRa Bio, Inc., Japan). For qRT-PCR analysis, each 20-µL reaction volume consisted of 1.0 µL of cDNA diluted by tenfold as a template with KOD SYBR qPCR Mix (Toyobo Co., Ltd., Japan) in accordance with the manufacturer's instructions. qRT-PCR was performed with a LightCycler 480 System (Roche Diagnostics, Switzerland) in accordance with the following cycling conditions: an initial denaturation step at 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s and at 55 °C for 31 s. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method with the 18S ribosomal RNA (*Ny18SrRNA*) gene as an internal reference. The relative expression level was calculated as a ratio of the mRNA level to the transcription level on day 0 of ACC treatment. All qRT-PCR analyses were performed in triplicate. The primers used in this study are listed in Table 1. The sequences of the analyzed genes were retrieved from the genome sequence data of *N. yezoensis* (Nakamura et al. 2013).

Statistical analysis

All data are expressed as the mean ± SD. The Mann–Whitney *U* test was used to identify statistically significant differences following treatment with and without plant hormones. For all analyses, $p < 0.05$ (significant) and $p < 0.01$ (highly significant) were considered thresholds of statistical significance.

Table 1 The list of primers used for gene expression analysis by quantitative real-time PCR

Primer name	Contig ID	Sequence (5′-3′)
NyALDH-F1	Contig_6867_g1587	TACCTGGGATTGGAA AGCTG
NyALDH-R1	Contig_6867_g1587	CCAATGAACAGCACA TGGTC
NyPOD-F1	Contig_27674_g6814	GCACGTACGGCTACC ACAC
NyPOD-R1	Contig_27674_g6814	CGACGACAATACCCA CATCC
NyBCS1L-F1	Contig_11756_g2797	AAGGAGGTGAAGCGT GATGA
NyBCS1L-R1	Contig_11756_g2797	GGGGGCATACAGGAA AAATG
NyHLIP-F1	Contig_21247_g5217	CTTTGTCGGCTCTGC TGTT
NyHLIP-R1	Contig_21247_g5217	GGACTGCGCGTTGATCTT
Ny18S-F1	*D79976	AGGGTTGATCCGCAG GGAAG
Ny18S-R1	*D79976	GCTTGCGCCCACTCC ATTAG

*Accession number in GenBank

Results

Neopyropia yezoensis gametophytes cultured at 15 °C were subjected to heat stress (28 °C) after treatment with SA, ABA, or ACC to assess the role of plant hormones in heat tolerance. After heat stress treatment, spots of discoloration, which indicate a drastic decrease in photosynthetic pigments, were observed in *N. yezoensis* pretreated with DMSO (control), 50 µM SA, or 50 µM ABA, whereas pretreatment with 50 µM ACC mitigated the discolored spots (Fig. 1). In response to heat stress, gametophytes pretreated with DMSO, SA, or ABA had cells with yellow-green chloroplasts, enlarged cells or dead cells. In contrast, pretreatment with ACC prevented the formation of cells with yellow-green chloroplasts or enlarged cells (Fig. 1).

Maximum quantum efficiency (F_v/F_m) using pulse amplitude modulation techniques was employed to assess the impact of heat stress on the photosynthetic capacity in *N. yezoensis*. The F_v/F_m value of gametophytes treated with DMSO, SA, or ABA ranged from 0.17 to 0.23, whereas that of gametophytes supplemented with ACC was 0.47 (Fig. 2).

Next, the effects of different concentrations of ACC against heat stress in gametophytes supplemented with ACC were compared. There was a significant difference in the F_v/F_m values of gametophytes pretreated with and without ACC (Fig. 3). The F_v/F_m value of gametophytes pretreated with a lower concentration of ACC (5 µM) slightly decreased as compared with those treated with higher concentrations (25 or 50 µM) in response to heat stress.

The effects of the ACC analogs ACBC and AIB on heat stress tolerance in gametophytes also were investigated. The F_v/F_m values of thalli treated with 5 or 50 µM ACC or 50 µM ACC analogs were relatively high in response to heat stress (5 µM ACC, 0.33; 50 µM ACC, 0.47; 50 µM ACBC, 0.46; 50 µM AIB, 0.46) (Fig. 4). However, there was no significant difference in the F_v/F_m values of thalli treated with 5 µM ACBC or 5 µM AIB as compared to the control (Fig. 4). These results indicate that the ACC analogs were less effective than ACC, and ACC enhanced heat stress tolerance independent of ethylene.

To understand the role of ACC treatment in acquired thermotolerance, the expression profiles of genes involved in stress responses were examined (Fig. 5). Based on our previous RNA-seq data in response to ACC (Uji et al. 2016), four candidate genes associated with thermotolerance were selected (Table 2). The mRNA levels of *NyALDH* (encoding aldehyde dehydrogenase), *NyHLIP* (encoding high light-inducible protein), and *NyPOD* (encoding haem peroxidase) had gradually increased after ACC treatment. The exogenous application of ACC resulted in the upregulation of *NyBCS1L* (encoding BCS1-like ATPase) to similar levels at 3 and 7 days after ACC treatment.

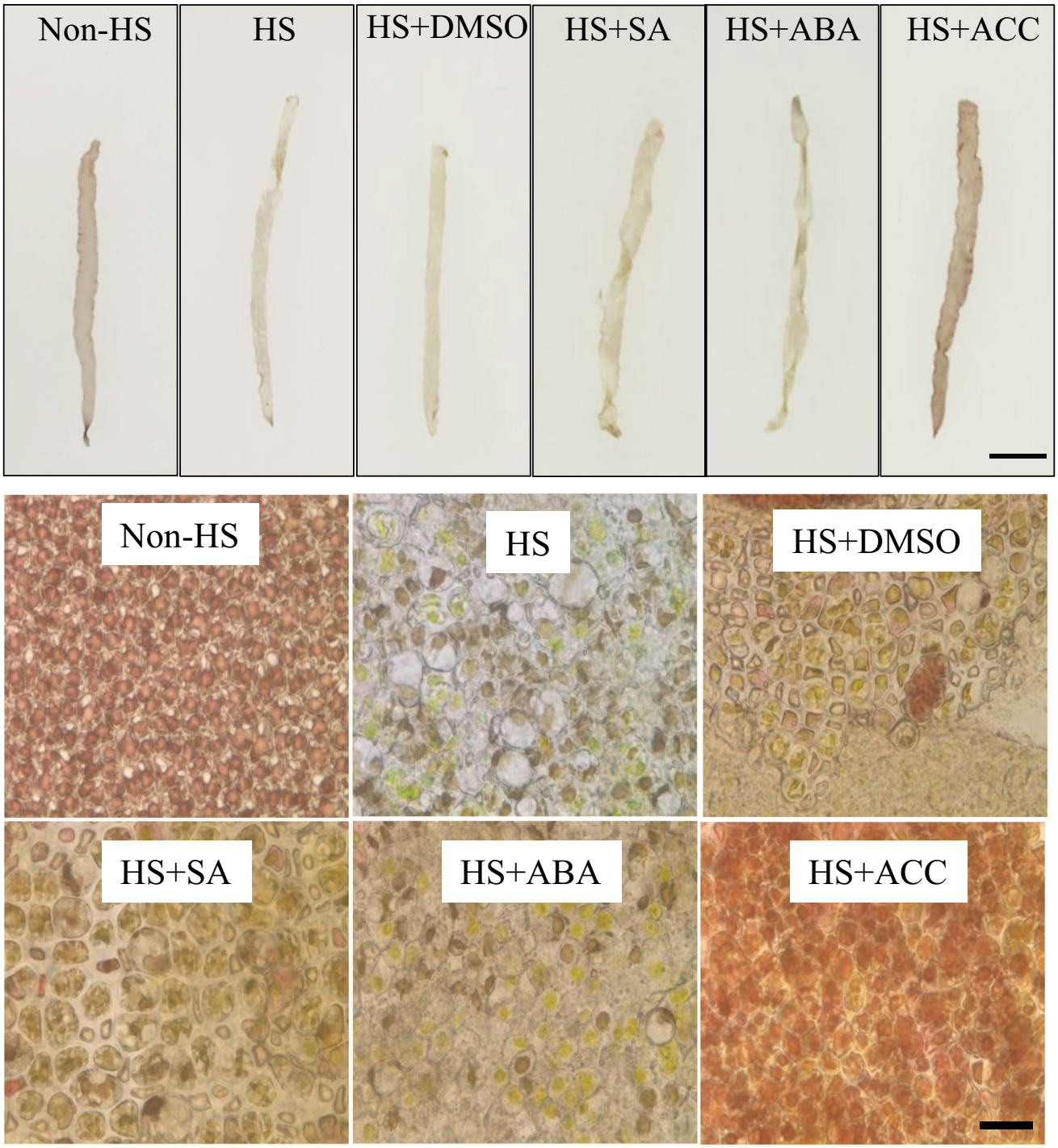

Fig. 1 Evaluation of exogenous applied plant hormones for heat stress tolerance in *N. yezoensis*. Gametophytes were subjected to heat stress at 28 °C for 2 weeks after 1 week of pretreatment with 0 (control), 0.05% DMSO (mock), 50 μM ABA, 50 μM SA, or 50 μM ACC. Gametophytes grown at 15 °C were used as controls (Non-HS). Scale bar = 100 mm (upper panel), 50 μm (lower panel)

Discussion

In the present study we showed that pretreatment with ACC for 1 week can mitigate the negative impacts of heat stress in *N. yezoensis* thalli. Thus, exogenous application of ACC to nori seedlings before setting seeding nets in bays and inland seas could significantly increase the yields under climate change. However, previous studies by our group showed that ACC treatment inhibited the growth of *N. yezoensis* (Uji et al. 2016, 2019). Organisms have evolved diverse

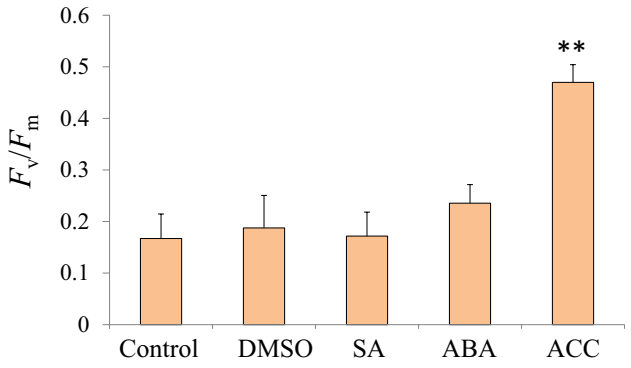

Fig. 2 Effect of exogenous plant hormones on the photosynthetic capacity in *N. yezoensis* under heat stress conditions. Maximum photochemical efficiency (F_v/F_m) was assessed in gametophytes subjected to heat stress at 28 °C for 2 weeks after 1 week of pretreatment with 0 (control), 0.05% DMSO (mock), 50 µM ABA, 50 µM SA, or 50 µM ACC. Data are expressed as the mean ± SD of five thalli for each condition. Double asterisks indicate significant differences at $p < 0.01$ between controls and treatments

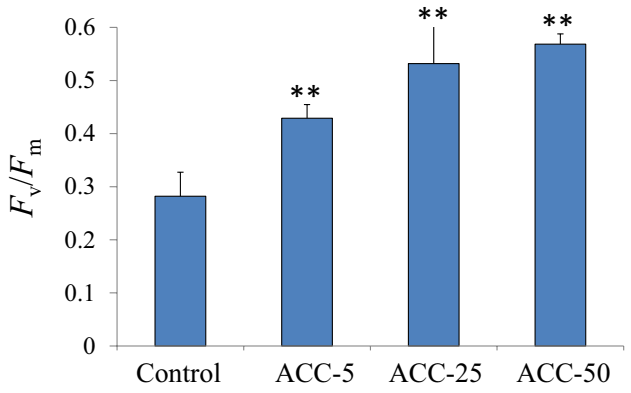

Fig. 3 Evaluation of different concentrations of exogenous ACC on the photosynthetic capacity of *N. yezoensis* under heat stress conditions. Maximum photochemical efficiency (F_v/F_m) was assessed in gametophytes subjected to heat stress at 28 °C for 2 weeks after 1 week of pretreatment with 0 (control), 5, 20 or 50 µM ACC. Data are expressed as the mean ± SD of five thalli for each condition. Double asterisks indicate significant differences at $p < 0.01$ between controls and treatments

mechanisms of "tradeoff" that enable the allocation of resources for growth to adapt life-threatening stress (Takatsuji 2017). Elucidating the mechanisms mediating tradeoffs between the stress tolerance and the growth under ACC signaling should be necessary to apply ACC as a tool in nori aquaculture.

Phytohormones are endogenous signaling molecules that play important roles in various aspects of plant development, growth, and stress responses (Gray 2004; Verma et al. 2016; Yu et al. 2020). Many studies have found that exogenous application of phytohormones, such as ABA and SA, significantly ameliorated heat-induced damage and improved heat tolerance in land plants (Devireddy et al. 2021; Li et al. 2021). Although current knowledge of plant hormone-mediated stress tolerance in algae remains limited, SA is reported to alleviate the adverse effects of high-temperature stress in the red alga *Gracilariopsis lemaneiformis* (Wang et al. 2017). In the current study, SA and ABA had no effect on heat stress tolerance in *N. yezoensis*. In contrast, the exogenous application of ACC as well as the analogs ACBC and AIB induced the acquisition of heat stress tolerance. In addition to classical phytohormones, recent studies have proposed that ACC, the direct precursor of the plant hormone ethylene, acts as a signaling molecule to regulate development and growth independent of ethylene biosynthesis in land plants and red algae (Van de Poel 2020). This report is the first to describe the involvement of ACC signaling in tolerance to heat stress of land plants and algae, independent of ethylene signaling.

Heat stress enhances production of ROS (superoxide [O_2–], hydroxyl radicals [OH^-], hydrogen peroxide [H_2O_2], and singlet oxygen [$^1O_2$]), which leads to oxidative damage of proteins, nucleic acids, and lipids, and eventual disruption of cellular homeostasis (Suzuki and Mittler 2006; Awasthi et al. 2015). For ROS detoxification, plants produce antioxidants, such as ascorbic acid (AsA) and glutathione, as well as ROS-scavenging enzymes (Choudhury et al. 2017), such as ascorbate peroxidase (APX), which is among the most important antioxidant enzymes in land plants (Shigeoka et al. 2002). Our previous study showed that ACC increased AsA synthesis in *N. yezoensis* gametophytes, while the expression levels of three APX genes of *N. yezoensis* were only slightly increased or decreased in the thalli after ACC treatment (Uji et al. 2020). In the present study, *NyPOD* transcripts had accumulated in gametophytes after ACC treatment, implying that NyPOD may play an important role as a ROS-scavenging enzyme during abiotic stress in *N. yezoensis*.

Excessive ROS accumulation also induced the production of aldehydes, which can cause genotoxic effects, such as lipid peroxidation, resulting in the loss of membrane integrity and subsequent cellular and developmental arrest (Kotchoni et al. 2006; Stiti et al. 2011). Thus, genomes of organisms encode aldehyde dehydrogenase (ALDH) enzymes that catalyze the oxidation of various aldehydes to carboxylic acids, thereby reducing lipid peroxidation (Stiti et al. 2011). Increased fluidity of the thylakoid membranes at high temperature causes light-harvesting complexes, located in the core of photosystem II (PSII), to become dislodged from the thylakoid membrane (Mathur et al. 2014). Previous studies of gain- and loss-of-function mutations in land plants suggest that ALDH enzymes reduce lipid peroxidation of thylakoid membranes under oxidative stress conditions (Kotchoni et al. 2006; Zhao et al. 2017). In addition, ALDH protein expression was elevated in *N. yezoensis* under high

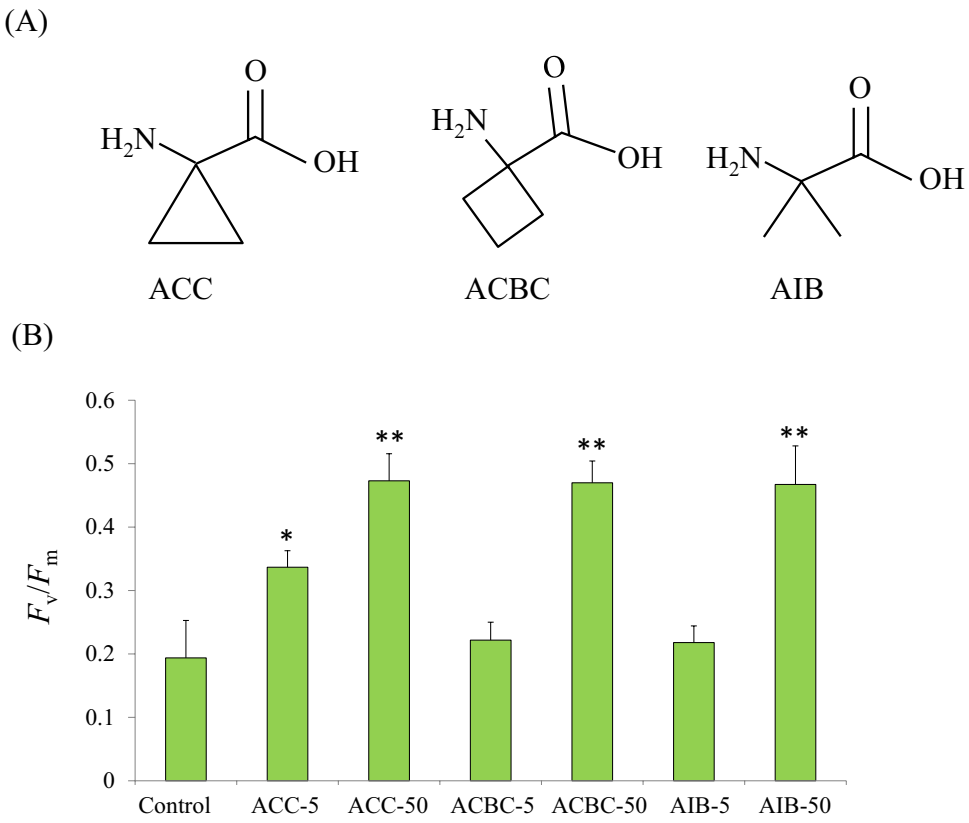

Fig. 4 Comparison of effects of exogenous ACC and analogs on the photosynthetic capacity of *N. yezoensis* under heat stress conditions. **A** Structural formulas of ACC and the analogs used in this study. **B** Maximum photochemical efficiency (F_v/F_m) was assessed in gametophytes subjected to heat stress at 28 °C for 2 weeks after 1 week of pretreatment with 0 (control), 5, 50 μM ACC, ACBC, or AIB. Data are expressed as the mean ± SD of five thalli for each condition. Asterisks and double asterisks indicate significant differences at $p < 0.05$ or 0.01, respectively, between controls and treatments

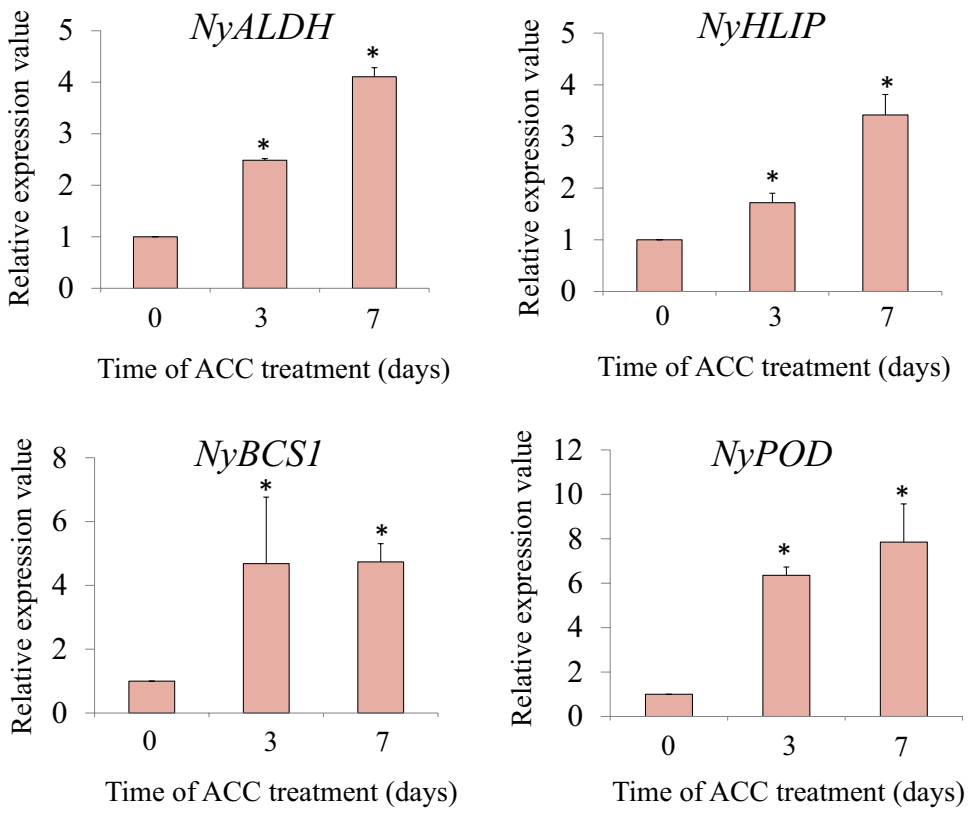

Fig. 5 Relative expression levels of genes associated with stress tolerance of *N. yezoensis* in response to ACC. RNA samples were prepared from gametophytes treated with 50 μM ACC for 0, 3, or 7 days, and expression levels were normalized to *Ny18SrRNA*. The results are presented as relative expression as compared to non-treated gametophytes (day 0). The data are presented as the mean ± SD of three independent experiments. Asterisks indicate significant differences at $p < 0.05$ between controls and treatments

Table 2 The list of tested genes for ACC response in *N. yezoensis*

Contig ID	Abbreviation	Functional categories	Description
Contig_6867_g1587	ALDH	Aldehyde scavengers	Aldehyde dehydrogenase
Contig_27674_g6814	POD	Oxidation/reduction reaction	Haem peroxidase
Contig_11756_g2797	BCS1L	Mitochondria chaperone	BCS1-like ATPase
Contig_21247_g5217	HLIP	Photoprotection of photosystem II	High light inducible protein

temperature and H_2O_2 stress conditions, and recombinant NyALDH enhanced salinity and oxidative stress tolerance in *Escherichia coli* (Lee and Choi 2018). In this study, the application of ACC increased *NyALDH* expression, suggesting that detoxification of aldehydes is important to protect peroxidation of lipid membranes to maintain photosynthetic capacity under heat stress conditions.

Photosynthesis is among the most heat-sensitive physiological processes because chloroplast is the primary sites of ROS production in response to heat stress (Hu et al. 2020). ROS, which are produced by PSI and PSII as well as the Calvin–Benson cycle, can cause irreversible oxidative damage to plant cells subjected to heat stress (Suzuki et al. 2012; Wang et al. 2018). In the present study, *N. yezoensis* cells subjected to heat stress contained yellow-green chloroplasts and the F_v/F_m value was decreased. However, ACC pretreatment mitigated these changes. As described above, ACC treatment greatly increased the AsA content of *N. yezoensis* thalli (Uji et al. 2020). AsA plays a protective role in photoinactivation by serving as a PSII electron donor, which can alleviate photodamage to PSII reaction centers caused by the accumulation of ROS under abiotic stress, including heat stress, conditions (Tóth et al. 2009, 2011). Thus, an increase in AsA during ACC treatment may retard the photoinactivation of PSII under heat stress in *N. yezoensis*, thereby alleviating damage to the photosynthetic machinery. Furthermore, exogenous application of ACC increased the *NyHLIP* transcripts encoding a homolog of HLIP of cyanobacteria, which are similar to light-harvesting chlorophyll a/b-binding proteins (Komenda and Sobotka 2016). Transcript levels of cyanobacterial HLIPs increase in response to high light conditions (Dolganov et al. 1995; He et al. 2001) and play a protective role against generation of singlet oxygen to prevent photoinactivation of PSII (Sinha et al. 2012; Komenda and Sobotka 2016). In a previous report, *NyHLIP* transcripts were upregulated under high irradiation and low temperature conditions, suggesting that NyHLIP functions not only in response to excess light but also to protect the cell against other stressors (Kong et al. 2012). In this study, the activation of NyHLIP in response to ACC may have also contributed to protect the photosynthetic apparatus against heat stress.

In addition to chloroplasts, the mitochondria are also main targets of oxidative damage under abiotic stress conditions (Bartoli et al. 2004). A previous study reported that *BCS1* mRNA levels of *Arabidopsis*, an ortholog of yeast *BCS1* involved in biogenesis of the cytochrome bc_1 complex (Nobrega et al. 1992), increased in response to SA and H_2O_2 (Ho et al. 2008). Similarly, in the present study, exogenous application of ACC upregulated *NyBCS1* expression, suggesting that BCS1 is a key to protect mitochondria against abiotic stress in both red and green lineage. Hence, future studies are warranted to elucidate the role of BCS1 in the stress response.

As mentioned above, ROS can cause oxidative damage to cells during environmental stress. However, ROS also play a key role as signal transduction molecules in plants by mediating various stress responses through interactions with hormonal signaling (Mittler et al. 2004; Torres and Dangl 2005; Suzuki et al. 2013). In signal transduction-associated ROS, respiratory burst oxidase homolog (*Rboh*) genes, encoding nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, are the main producers of ROS (Mittler et al. 2004; Torres and Dangl 2005). Loss of function of NADPH oxidase is reported to impair the heat stress response in *Arabidopsis* (Larkindale et al. 2005; Miller et al. 2009). In Bangiales, the formation of sporophytes (also called as the conchocelis phase), which grow in the summer, produces significantly higher levels of H_2O_2 accompanied with higher activities of NADPH oxidase as compared to gametophytes, which usually grow in the winter (Luo et al. 2014). In addition, the induction of H_2O_2 and mRNA levels of *Rboh* was observed in Bangiale species under high-temperature conditions (Luo et al. 2014). The results of our previous study revealed that ROS generation in *N. yezoensis* gametophytes treated with ACC was accompanied by an increase in *Rboh* transcripts (Uji et al. 2020), implying that fine-tuning the balance of ROS by ACC treatment contributed to prevent oxidative damage caused by heat stress in *N. yezoensis* gametophytes.

Heat shock proteins (HSPs), which function as molecular chaperones in maintaining homeostasis in protein folding, are assumed to play a central role in acquired thermotolerance in plants (Wang et al. 2004; Kotak et al. 2007). To date, five families of HSPs have been defined based on molecular sizes: Hsp100, Hsp90, Hsp70, Hsp60, and small HSPs (sHSPs/HSP20). In addition to HSPs, multiprotein bridging factor 1 (MBF1), which functions as a non-DNA-binding transcription co-factor or a bona fide transcription factor, plays an important role in response to abiotic stressors,

particularly heat stress, in land plants (Suzuki et al. 2011; Jaimes-Miranda and Chávez Montes 2020). Although there is limited evidence of thermotolerance mechanisms in seaweed, the mRNA levels of *NysHSPs* and *NyMBF1* were increased in *N. yezoensis* gametophytes under heat stress conditions (Uji et al. 2013, 2019). On the other hand, the transcript levels of *NysHSPs* were only slightly increased in response to ACC treatment as compared to under heat stress conditions (Uji et al. 2019). Moreover, our RNA-seq data showed that *NyMBF1* was not identified as an ACC-responsive gene in *N. yezoensis*. These findings raise the possibility that ACC enhances heat tolerance in *N. yezoensis* via a somewhat different response to heat stress. Similarly, brassinosteroid-mediated thermotolerance is not necessary for the expression of HSPs in land plants (Kagale et al. 2007). Future comparisons of thermoprotective mechanisms acquired by ACC application and moderate heat stress will further the current understanding of the pathways that protect *N. yezoensis* against stress-induced damage.

Conclusion

The results of this study present evidence that ACC can serve as a priming hormone that enables *N. yezoensis* to resistant to heat stress. On the other hand, treatment with the plant hormones ABA and SA, which are regulators of the stress responses in land plants, failed to improve heat stress resistance in *N. yezoensis*. ACC can activate the expression of genes associated with antioxidant defense systems, such as *NyHLIP* and *NyBSC1*, to protect against impaired function of chloroplasts and mitochondria caused by heat-induced oxidative damage. A future challenge will be to elucidate the ACC-associated signaling pathways leading to heat stress tolerance in *N. yezoensis*, which could provide opportunities to generate thermotolerant varieties of Bangiales.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contribution TU was responsible for the design of the experiments and interpretation of the data. TU performed the experiments. TU and HM wrote the manuscript. All authors have read and approved the final version of the manuscript.

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Declarations

Conflict of interest The authors declare no competing interests.

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