α-aminoisobutyric acid mimics the effect of 1-aminocyclopropane-1-carboxylic acid to promote sexual reproduction in the marine red alga *Pyropia yezoensis* (Rhodophyta)

Harune Endo

Laboratory of Aquaculture Genetics and Genomics, Division of Marine Life Science, Faculty of Fisheries Sciences, Hokkaido University, Hakodate 041-8611, Japan

Hiroyuki Mizuta

Laboratory of Aquaculture Genetics and Genomics, Division of Marine Life Science, Faculty of Fisheries Sciences, Hokkaido University, Hakodate 041-8611, Japan

Toshiki Uji[1]

Laboratory of Aquaculture Genetics and Genomics, Division of Marine Life Science, Faculty of Fisheries Sciences, Hokkaido University, Hakodate 041-8611, Japan

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[1] Tel/Fax: +81-138-40-8864

E-mail: t-uji@fish.hokudai.ac.jp

17 Abstract

18 1-aminocyclopropane-1-carboxylic acid (ACC), a precursor for ethylene, stimulates the
19 switch from a vegetative to a sexual reproductive phase in the marine red alga *Pyropia*
20 species. This study explored the effects of ethylene biosynthesis inhibitors on the sexual
21 reproduction of gametophytes of the red alga *Pyropia yezoensis* to gain a functional
22 understanding of the role of ACC as a plant hormone in red algae. Here we show that
23 two inhibitors of ACC synthesis in higher plants, 2-aminoethoxyvinyl glycine (AVG)
24 and aminooxyacetic acid (AOA), had no effect on the growth and gametogenesis of *P.*
25 *yezoensis*. In contrast, exogenous application of α-aminoisobutyric acid (AIB), a
26 structural analog of ACC that blocks the conversion of ACC to ethylene in higher plants,
27 induced the formation of spermatangia and carpospores, in a similar manner as ACC,
28 without endogenous ACC accumulation. The treatment of AIB failed to inhibit ethylene
29 production in the gametophytes. The present results suggest that AIB mimics the effect
30 of ACC to induce the sexual reproduction and support our previous study that ACC has
31 a role in the regulation of the sexual reproduction independent from ethylene.

32

33 Keywords: 1-aminocylopropane-1-carboxylic acid, ethylene, plant hormone, *Pyropia*,
34 red algae, sexual reproduction

35 Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; ACS, ACC synthase;

36 ACO, ACC oxidase; AIB, α-aminoisobutyric acid; AOA, aminooxyacetic acid; AVG,

37 2-aminoethoxyvinyl glycine

38

39

40 Introduction

41 Plants produce hormones (also known as phytohormones or plant growth regulators)
42 that regulate plant growth, development, and environmental responses (Vanstraelen and
43 Benkova 2012; Xia et al. 2015; Verma et al. 2016). In plant hormone research, the use
44 of a wide variety of small molecules such as agonists, antagonists, and inhibitors of the
45 biosynthetic pathway and transports have significantly enhanced our understanding of
46 the molecular basis of hormone actions in higher plants (Fonseca et al. 2014; Rigal et al.
47 2014). In macroalgae, treatment with auxin efflux inhibitors, such as
48 naphthylphthalamic acid (NPA), elevates indole-3-acetic acid (IAA) accumulation and
49 reduces environmental polarization in response to gravity and light vectors in embryos
50 of *Fucus distichus*, a brown alga (Basu et al. 2002; Sun et al. 2004). Jasmonate (JA) is
51 related to cystocarp development in the red macroalga *Grateloupia imbricate*, and the
52 application of phenidone, a specific inhibitor of lipoxygenases (LOX) that catalyzes the
53 first step in the biosynthesis of JA, decreases the number of the cystocarps concomitant
54 with the repression of the JA release (Garcia-Jimenez et al. 2016). In addition, callus
55 induction from leaf explants of the brown alga *Sargassum horneri* was achieved when
56 grown in medium supplemented with uniconazole, a triazole-type inhibitor of
57 cytochrome P450 enzymes for gibberellin biosynthesis, trans-zeatin biosynthesis, and

58 abscisic acid catabolism (Uji et al. 2016b). However, in contrast to higher plants, few
59 reports are available on the use of small molecules involved in plant hormones in
60 macroalgal research, which hinders the elucidation of the functional roles of plant
61 hormones.

62 Ethylene, a simple gaseous plant hormone, is derived from methionine (Met),
63 which is first converted to S-adenosyl-methionine (SAM) by SAM synthetase. SAM is
64 then converted into 1-aminocylopropane-1-carboxylic acid (ACC), an ethylene
65 precursor (Yang and Hoffman 1984). This step is catalyzed by ACC synthase (ACS), an
66 aminotransferase that requires pyridoxal 5'-phosphate (PLP) as a cofactor. In the second
67 step, ACC is oxidized by an O_2-activating non-heme iron enzyme, ACC oxidase (ACO),
68 giving rise to ethylene, carbon dioxide (CO_2), and hydrogen cyanide. Ethylene
69 production can be manipulated by the inhibition of ACS and ACO activity (Fig. 1). For
70 instance, 2-aminoethoxyvinyl glycine (AVG) and aminooxyacetic acid (AOA) inhibit
71 the activity of ACS and consequently decrease ethylene production by preventing the
72 production of ACC (Amrhein and Wenker 1979; Boller et al. 1979; Yang and Hoffman
73 1984). Additionally, α-aminoisobutyric acid (AIB) is a structural analog of ACC that
74 blocks ACO activity by acting as a competitive inhibitor of the ACC substrate (Satoh
75 and Esashi 1980; 1982; Serrano et al. 1990). The pharmacological manipulation of

76 ethylene biosynthesis using these inhibitors has increased our understanding of the roles
77 of ethylene in higher plants (Tamimi and Timko 2003; Tian et al. 2014).

78 The marine red alga *Pyropia* (formerly *Porphyra*), a genus of Bangiophyceae, is a
79 significant marine crop that is harvested to produce the food “nori”. The heteromorphic
80 life history of *Pyropia* is comprised of a blade gametophyte and filamentous sporophyte
81 (Fig. 2). This genus forms male (spermatia) and female (carpogonia) gametes during
82 sexual reproduction. After fertilization, successive cell divisions occur to produce clones
83 of the zygote called carpospores that will grow into sporophytes. Our previous research
84 revealed that the exogenous application of ACC promoted gametogenesis and enhanced
85 the antioxidant capacity in accompany with ethylene emission in the monoecious *P.*
86 *yezoensis* (Uji et al. 2016a). Research on the dioecious species *P. pseudolinearis*
87 revealed that ACC can modulate the expression of genes involved in the regulation of
88 cell division and cell wall organization, which leads to the formation of spermatangia
89 and parthenosporangia in male and female gametophytes, respectively (Yanagisawa et al.
90 2019). Our recent study suggests that ACC acts as a signaling molecule independent
91 from ethylene in the regulation of sexual reproduction through alterations to the redox
92 state in *P. yezoensis* (Uji et al. 2020).

93 In the present study, we investigated the effects of three ethylene biosynthetic

pathway inhibitors on the sexual reproduction of *P. yezoensis* gametophytes to gain a better understanding of ACC plant hormone action in *Pyropia* species. The present results suggest that AIB mimics the effect of ACC to induce the sexual reproduction in *P. yezoensis*.

Materials and methods

Algal cultures

Standard cultivation of gametophytes of *P. yezoensis* strain TU-1 was conducted at 15 °C in glass flasks (150 mL volume) with 100 mL of medium. The medium consisted of autoclaved seawater enriched with sterile vitamin-free Provasoli solution (PES; Provasoli, 1968). Light was supplied by cool-white fluorescent lamps at 60 μmol photons m^{-2} s^{-1} irradiance with a photoperiod regime of 10 h light:14 h dark (short day condition) or 14 h light:10 h dark (long day condition).

Chemical treatments

The gametophytes of *P. yezoensis* were treated with the chemical compounds generally used as ethylene biosynthesis inhibitors in higher plants. Five individual immature gametophytes of ca. 20 mm blade length that were microscopically determined to bear

112 only vegetative cells were cultured in glass flasks (150 mL volume) with 100 mL PES
113 medium with either three inhibitors prepared each concentration: 0, 5, 50 μM
114 aminoethoxyvinylglycine (AVG) (Cayman Chemical Company, MI, USA); 50, 500 μM
115 α-aminoisobutyric acid (AIB) (Tokyo Chemical Industry, Tokyo, Japan); 50, 500 μM
116 aminooxyacetic acid (AOA) (FUJIFILM Wako Pure Chemical Corporation, Osaka,
117 Japan); or 50, 500 μM ACC (Tokyo Chemical Industry). Two pieces of glass (20 mm ×
118 25 mm) were placed on the bottom of the culture flask to capture the formation of
119 carpogonia, the female gametes. The gametophytes were cultured with the chemical
120 reagents for 7 d, then the number of gametophytes that had formed clusters of
121 spermatangia were counted under a Leica DM 5000 B microscope (Leica Microsystems,
122 Tokyo, Japan). In this study, a mature gametophyte was defined as a thallus bearing at
123 least five clusters of spermatangia because carpogonium from *P. yezoensis* are almost
124 indistinguishable from surrounding vegetative cells. In addition, after 14 d, the numbers
125 of discharged carpospores attached to the pieces of glass were counted under a
126 microscope as an index of formation of carpogonia. The gametophyte blade lengths
127 were measured after 10 d and growth rate was calculated as the mean percentage of
128 length increase per day using the following formula: Growth rate = $[100(BL_t - BL_0)/BL_0]/t$, where BL_0 = initial blade length, BL_t = blade length at days of culture, t =
129

130 culture time. The experiments under the long day or the short day culture were
131 conducted at three or eight replicates with 5 thalli each condition, respectively. In these
132 experiments, thalli treated without inhibitors were used as a control.
133
134 *Measurement of ethylene production*
135 To examine whether AIB has an inhibitory effect on ethylene production in
136 gametophytes, we measured ethylene levels after 10 d treatments with AIB. Vegetative
137 gametophytes (fresh weight: FW ca. 0.1 g) were cultured in 20 mL aluminum sealed
138 vials with butyl-gum septums (GL Science, Tokyo, Japan) with 10 mL of medium
139 containing 0, 500 μM ACC, or 500 μM ACC and AIB, at 15 °C under a 10 h light:14 h
140 dark photoperiod regime using cool-white fluorescent lamps at 60 μmol photons m^{-2} s^{-1}.
141 After 10 d, the samples were analyzed by GC analysis of the volatile compounds. For
142 the static headspace GC analysis, the sample vial was transferred into the HS-20
143 headspace auto-sampler (Shimadzu Corporation Kyoto, Japan) of the GC apparatus. The
144 headspace gas in the vial was automatically pressurized at 60 °C for 2 min and then
145 immediately injected through a loop into a GC-2014AFSC (Shimadzu) equipped with
146 an HP-1 capillary column (50 m length, 0.32 mm i.d. and 1.05 μm film thickness;
147 Agilent Technologies, CA, USA) and a flame ionization detector. An initial oven

148 temperature of 40 °C for 5 min was followed by heating at 3 °C/min to 70°C, then
149 20 °C/min to 200 °C, and finally, the temperature was held at 200 °C for 4 min. Both
150 the injection port and the flame ionization detector were set at 250 °C. The content of
151 ethylene was expressed in nmol day^{-1} g^{-1} FW. These experiments were repeated in
152 triplicate and thalli treated with ACC were used as a control.
153
154
155 *Measurement of ACC content*
156 ACC concentrations in the gametophytes treated with AIB were determined following
157 the method of Wächter et al. (1999) with minor modifications. The algal materials were
158 harvested, immediately frozen with liquid nitrogen, and stored at −80 °C until the
159 extraction of ACC. A 1.5 g sample (FW) was ground in liquid nitrogen with a mortar
160 and pestle, followed by homogenization in 7.5 mL 80% methanol with 2 mg/L
161 dibutylhydroxytoluene. The extract was stored at room temperature for 45 min, then
162 centrifuged for 15 min at 2000 × g at 20 °C. The extraction was repeated with 6 mL
163 80% methanol and the supernatants were evaporated to dryness with a Rotary
164 Evaporator N-N/NVC-1100 (EYELA, Osaka, Japan) at 40 °C. The dry residue was
165 dissolved in 2 mL distilled water and 4 mL dichloromethane, then centrifuged at 2000 ×

166 g at 20 °C for 5 min. The conversion of ACC to ethylene was performed following the
167 procedure described by Lizada and Yang (1979). A 1.6 mL aliquot of the upper aqueous
168 phase was mixed with 0.1 mL $HgCl_2$ (20 mM) in a 20 mL aluminum sealed vial with a
169 butyl-gum septum. NaOCl-solution (5% NaOCl and 50% NaOH (2:1, v/v)) was injected
170 through the septum with a syringe. The vials were shaken, incubated for 5 min and then
171 the sample was assayed for ethylene by GC analysis according to the method described
172 above. The ACC content was expressed as nmol g^{-1} FW. These experiments were
173 repeated in triplicate and thalli treated without ACC/AIB were used as a control.
174
175 *Statistical analysis*
176 Data are expressed as mean ± standard deviation (SD). The results of treatments with
177 and without inhibitors of the ethylene biosynthetic pathway were analyzed using
178 Mann-Whitney's U test. For all analyses, $p < 0.05$ or 0.01 were considered statistically
179 significant.
180
181 Results
182 *Effects of ethylene inhibitors on growth and gametogenesis*
183 When the gametophytes were cultured with 5, 50 μM AVG or 5, 50 μM AOA under the

184 long day culture condition that promotes the sexual reproduction, there were no
185 significant differences in spermatangia formation and growth rates between thalli
186 treated with and without the inhibitors (Table 1). These results imply that ACS
187 inhibitors generally used in higher plants are ineffective in the gametophytes. Contrary
188 to expectations, gametophytic thalli treated with 50 μM or 500 μM AIB formed
189 significantly more spermatangia clusters and gametophytes cultured with 500 μM AIB
190 exhibited slight growth repression compared to the thalli in the absence of chemical
191 compounds (control) (Table 1).
192
193 *Comparison of the effects of AIB and ACC on induction of sexual reproduction*
194 The results in the effects of AIB prompted us to compare with the induction level of
195 AIB and ACC on the sexual reproduction in the gametophytes. The gametophytes were
196 cultured with 50, 500 μM ACC or 50, 500 μM AIB under the short day culture
197 condition that represses the sexual reproduction. Treatment with 500 μM AIB promoted
198 the formation of spermatangia on the upper parts of the thalli, whereas 500 μM ACC
199 promoted spermatangia formation on the upper to middle sections (Fig. 3). All of the
200 gametophyte thalli that were cultured with 50, 500 μM ACC or 50, 500 μM AIB formed
201 spermatangia, whereas only 22.5% of thalli formed spermatangia when the

202 gametophytes were cultured without chemical compounds (control) (Fig. 4a). The
203 growth rate of gametophytes cultured in medium containing 50 and 500 μM ACC
204 exhibited 14.6% and 13.7%, respectively, whereas that of gametophytes grown with 50
205 and 500 μM AIB exhibited 20.2% and 12.2%, respectively (Fig. 4b). The number of
206 discharged carpospores from the gametophytes treated with ACC or AIB was
207 significantly higher than the control, but AIB has less effective than ACC (Fig. 4c).
208 These results indicate that AIB can induce the sexual reproduction in a similar manner
209 as ACC.
210
211 *Evaluation of AIB as an inhibitor of ACC to ethylene conversion*
212 The ACC contents of thalli treated with AIB were investigated to consider the
213 possibility that AIB promoted the sexual reproduction through endogenous ACC
214 accumulation by blocking the conversion of ACC to ethylene in the gametophytes. As
215 shown in Fig.5, exogenous application of 50 and 500 μM ACC increased the contents of
216 ACC *in vivo*, whereas no accumulation of ACC was confirmed in thalli supplemented
217 with 500 μM AIB. These results indicated that AIB can induce the sexual reproduction
218 without endogenous ACC accumulation and raised the possibility that AIB is ineffective
219 inhibitor of ACO. To confirm this, the ethylene production was examined in thalli

220 treated with AIB. Since our previous study revealed small amount of ethylene release in
221 the gametophytes under normal conditions (Uji et al. 2016a), we used thalli increased
222 ACC/ethylene *in vivo* by supplement with 500 μM ACC as a control. The results
223 showed that there were no significant differences in ethylene production between the
224 thalli treated without AIB and with 0.5 mM AIB (Fig.6). In addition, no inhibition of
225 ethylene production in the gametophytes treated with the even high doses of AIB (10
226 mM AIB) (Fig.6) which the concentration strongly inhibits the conversion of ACC to
227 ethylene in higher plants (Satoh and Esashi 1982; Serrano et al. 1990).
228
229 Discussion
230 Our recent study of *P. yezoensis* suggests that ACC acts as a signaling molecule
231 independent of ethylene signaling in the regulation of sexual reproduction through
232 alterations to the redox state (Uji et al. 2020). Interestingly, previous studies also
233 indicate that ACC regulates plant development in the higher plant *Arabidopsis thaliana*,
234 independent of its role as an ethylene precursor (Vanderstraeten et al. 2019; Polko and
235 Kieber 2019). Based on these findings, Vanderstraeten et al. (2019) suggest that AIB
236 may have the capacity to interact with ACC binding proteins (e.g., putative ACC
237 receptors), because AIB is structurally similar to ACC. Supporting this possibility, the

238 present study revealed that AIB promoted the formation of spermatangia and
239 carpospores in gametophytes without endogenous ACC accumulation in a similar
240 manner as ACC. However, the comparative experiment between ACC and AIB
241 treatments revealed that AIB promoted the formation of spermatangia on the upper parts
242 of the thalli, whereas ACC promoted spermatangia formation on the upper to middle
243 sections (Fig. 3). In addition, the number of discharged carpospores in the gametophytes
244 treated with AIB was smaller than that of ACC (Fig. 4).These results suggest that AIB
245 binds on the same target of ACC such as a putative ACC receptor to act ACC signaling,
246 but the binding capacity of AIB may be weaker than that of ACC in *P. yezoensis*. Future
247 work is needed to identify ACC receptor(s) and ACC signaling pathway(s) to uncover
248 the mechanism regulating the sexual reproduction in *Pyropia*.
249 Recent genetic analyses of mutants that affect biosynthesis or plant hormone
250 responses have revealed a number of signaling pathways in higher plants (Browse 2009;
251 Zhao and Li 2012). To elucidate the role of ACC, the analysis of ACC response to
252 ethylene-insensitive mutant contributed to the understanding of the ACC dependent
253 pathway (Tsuchisaka et al. 2009; Tsang et al. 2011). However, a rapid and simple
254 method of obtaining stable mutants has not yet been established for red macroalgae.
255 Thus, knowledge about the effects of small molecules that act as agonists, antagonists,

256 and inhibitors of biosynthetic pathways for plant hormones is indispensable to advance
257 macroalgal plant hormones research. In the previous study, exogenous application of
258 ACC induced ethylene emission in *P. yezoensis*, indicating that the effect of ACC
259 treatment is indistinguishable between the ACC- and ethylene-dependent pathways (Uji
260 et al. 2016). In contrast, AIB mimics the effect of ACC in *P. yezoensis* gametophytes
261 and thereby discriminates between ACC- and ethylene-responses, indicating that AIB
262 could provide a useful approach to elucidate the ACC-dependent, and
263 ethylene-independent pathways in red algae.

264 Genome sequence analyses have revealed that the homolog of the ACO gene is
265 absent in the genomes of red algae, including *P. yezoensis*. Consistent with this, AIB
266 application failed to inhibit ethylene production in the gametophytes (Fig. 6). In
267 addition to the absent ACO gene, there is no homolog for the receptors and components
268 of the ethylene signaling pathway in the red algal genome. Consistent with these
269 findings, our recent study revealed that exogenous application of ACC promoted the
270 formation of spermatia and carporspores in the gametophytes, whereas ethephon, an
271 ethylene-releasing compound, did not stimulate sexual reproduction (Uji et al. 2020). In
272 addition to the red algal genome, putative homologs for the perception and signaling of
273 ethylene were apparently absent in chlorophyte green algae. This finding is supported

by a lack of ethylene-binding activity in two chlorophytes, *Chlamydomonas reinhardtii* and *Acetabularia acetabulum* (Wang et al. 2006). In contrast, the charophyte green alga *Spirogyra pratensis* induced cell elongation in response to ethylene and possesses functionally conserved homologs of a complete set of ethylene-signaling genes (Ju et al. 2015). These findings suggest that the origin of ethylene response and signaling in plants occurred during the evolution of the charophyte lineage, prior to the colonization of land (Ju et al. 2015). However, exogenous application of ethylene promoted tetrasporogenesis in the red marine alga *Pterocladiella capillacea* (Garcia-Jimenez and Robaina 2012) and regulated gene expression during carposporogenesis in the red marine alga *Grateloupia imbricate* (Garcia-Jimenez et al. 2018). Hence, the physiological roles of ethylene in red algae require further investigation.

In the present study, treatment with ACS inhibitors generally used in higher plants, such as AVG and AOA did not have a significant effect on sexual reproduction in *P. yezoensis*. On the other hand, exogenous application of AIB induced the formation of spermatangia and carpospores without endogenous ACC accumulation, suggesting that AIB have the capacity to interact with ACC binding proteins to induce the sexual reproduction. This study supports that ACC acts as a plant hormone independent of ethylene in *Pyropia*. Further studies on the identification of ACC receptor(s) and ACC

292 signaling pathway(s) could contribute to elucidate the role of ACC beyond the precursor
293 of ethylene in red algae.
294
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296
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Figure legends

Figure 1. The ethylene biosynthetic pathway and the inhibitors used in this study. Met, Methionine; SAM, S-adenosyl-methionine; ACC, 1-aminocyclopropane-1-carboxylic acid; AVG, 2-aminoethoxyvinyl glycine; AOA, aminooxyacetic acid; AIB, α-aminoisobutyric acid

Figure 2. Life cycle of *Pyropia* species

The macroscopic leafy gametophytes of *Pyropia*, which are harvested to produce the food for nori or laver, bear non-motile male (spermatia) and female (carpogonia)

gametes on the thallus during sexual reproduction. Fertilization occurs when the female gametes are still retained on the gametophytes and successive cell divisions produce clones of the zygote (carpospores), which develop into microscopic filamentous sporophytes referred as the conchocelis. The mature sporophytes produce conchospores that are released from conchosporangia and settle on the substratum, where they germinate to form new gametophytes. Some *Pyropia* such as *P. yezoensis* can also reproduce asexually in the gametophytes by asexual spores called archeospores.

Figure 3. Effect of α-aminoisobutyric acid (AIB) on sexual reproduction in *Pyropia yezoensis* gametophytes under the short day culture condition. Gametophytes were cultured in media containing 0 or 500 μM ACC, or 500 μM AIB. The thalli treated with ACC and AIB formed spermatangia, which were clear or discolored. Scale bar = 10 mm.

Figure 4 α-aminoisobutyric acid (AIB) mimics the effect of ACC on the sexual reproduction in *Pyropia yezoensis* gametophytes under the short day culture condition. (a) Formation of spermatangia clusters on gametophytes after 7 d treatment with ACC or AIB. (b) Growth rate of gametophytes after 10 d treatment with ACC or AIB. (c) The

number of carpospores released from gametophytes after 14 d treatment with ACC or AIB. Data are expressed as mean ± SD. of eight independent experiments with five thalli for each condition. Asterisks and double asterisks indicate significant differences at $p < 0.05$ and $p < 0.01$, respectively, between control and treatments.

Figure 5. Effect of α-aminoisobutyric acid (AIB) on ACC accumulation in *Pyropia yezoensis* gametophytes. Gametophytes were cultured in media containing 0 (control) or 50, 500 μM ACC, or 500 μM AIB. The ACC production is expressed as mean ± SD (nmol g^{-1} fresh wt.).

Figure 6. Effect of α-aminoisobutyric acid (AIB) on ethylene production in *Pyropia yezoensis* gametophytes treated with ACC. The ethylene production is expressed as mean ± SD (nmol day^{-1} g^{-1} fresh wt.).

Table 1 Effect of ethylene biosynthetic pathway inhibitors on spermatangenesis and growth rate in *Pyropia yezoensis* gametophytes under the long day culture condition

Treatments (μM)	% of thallus with cluster of spermatangia	Elongation rate (mean ± SD% d^{-1})
Control (No treatment)	33.3 ± 21.6	17.3 ± 1.2
AVG 5	40.0 ± 37.4	12.5 ± 5.2
AVG 50	33.3 ± 29.4	11.9 ± 1.5
AOA 5	33.3 ± 16.3	15.2 ± 3.0
AOA 50	66.7 ± 8.2	13.5 ± 0.7
AIB 50	100.0 ± 0.0 *	12.8 ± 2.1
AIB 500	100.0 ± 0.0 *	8.4 ± 5.7

Data are expressed as means ± SD of three independent experiments with 5 thalli each condition
Asterisks indicate significant differences at $p < 0.05$ between control and treatments.
Elongation rate = $[100(BL_t - BL_0)/BL_0]/t$, BL_0 = initial blade length, BL_t = blade length at 10 days culture, t = culture time.

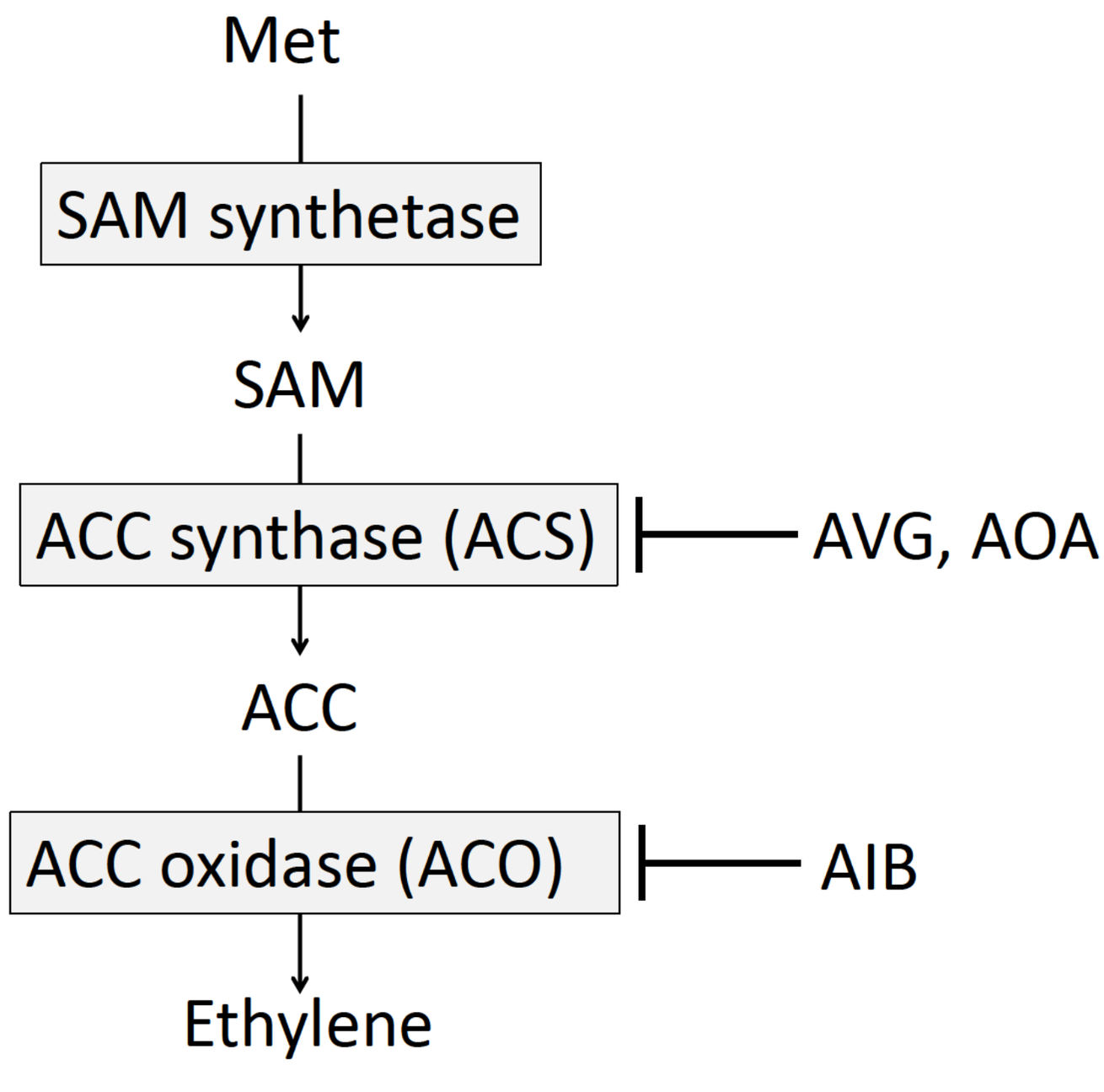
Met
SAM synthetase
SAM
ACC synthase (ACS)
AVG, AOA
ACC
ACC oxidase (ACO)
AIB
Ethylene

Fig. 1

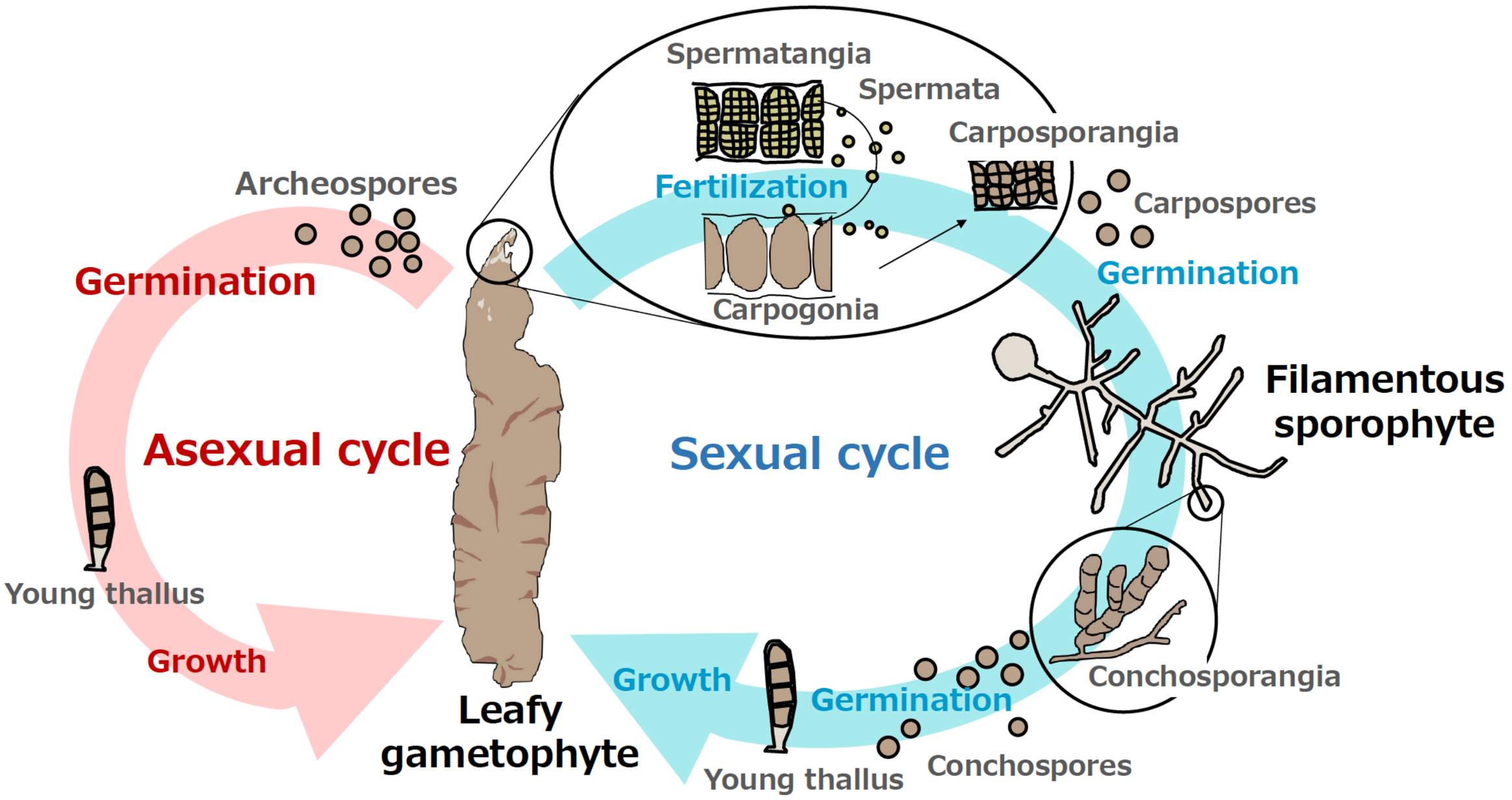
Spermatangia
Spermata
Carposporangia
Fertilization
Archeospores
Carpospores
Germination
Carpogonia
Germination
Filamentous sporophyte
Asexual cycle
Sexual cycle
Young thallus
Growth
Growth
Leafy gametophyte
Germination
Conchosporangia
Young thallus
Conchospores

Fig. 2

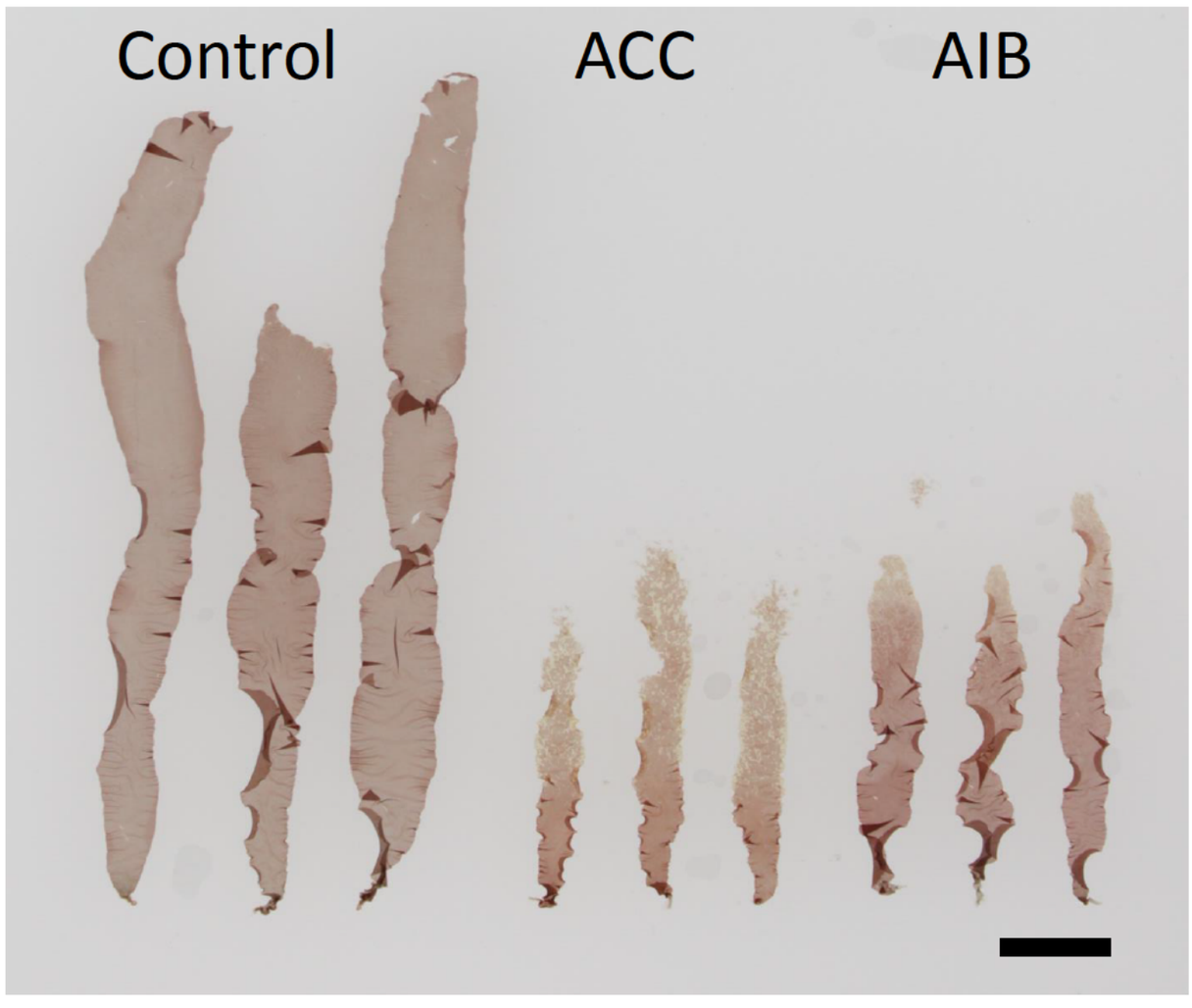

Fig. 3

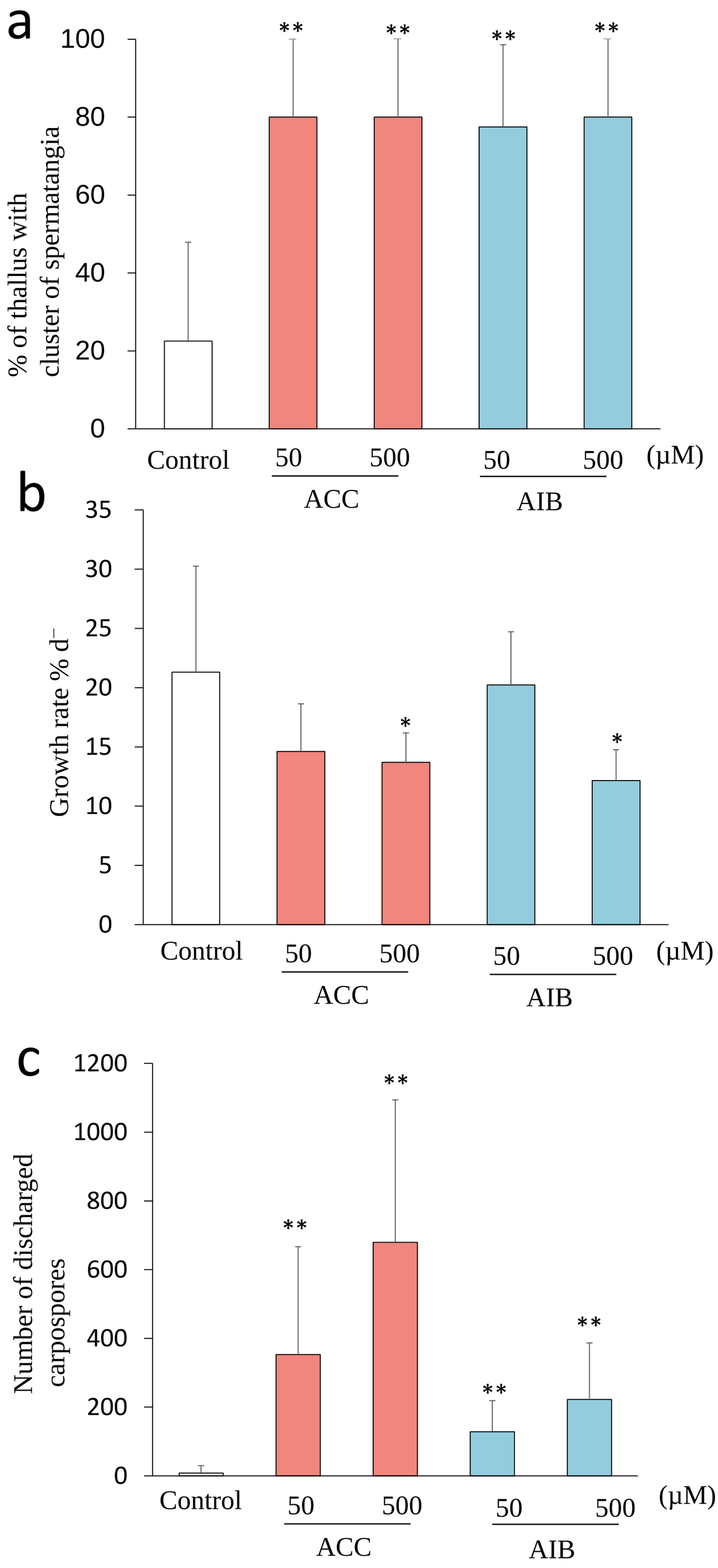
a
100
80
60
40
20
0
% of thallus with cluster of spermatangia
**
**
**
**
Control
50
500
50
500
(µM)
ACC
AIB
b
35
30
25
20
15
10
5
0
Growth rate % d⁻
*
*
Control
50
500
50
500
(µM)
ACC
AIB
c
1200
1000
800
600
400
200
0
Number of discharged carpospores
**
**
**
**
Control
50
500
50
500
(µM)
ACC
AIB

Fig. 4

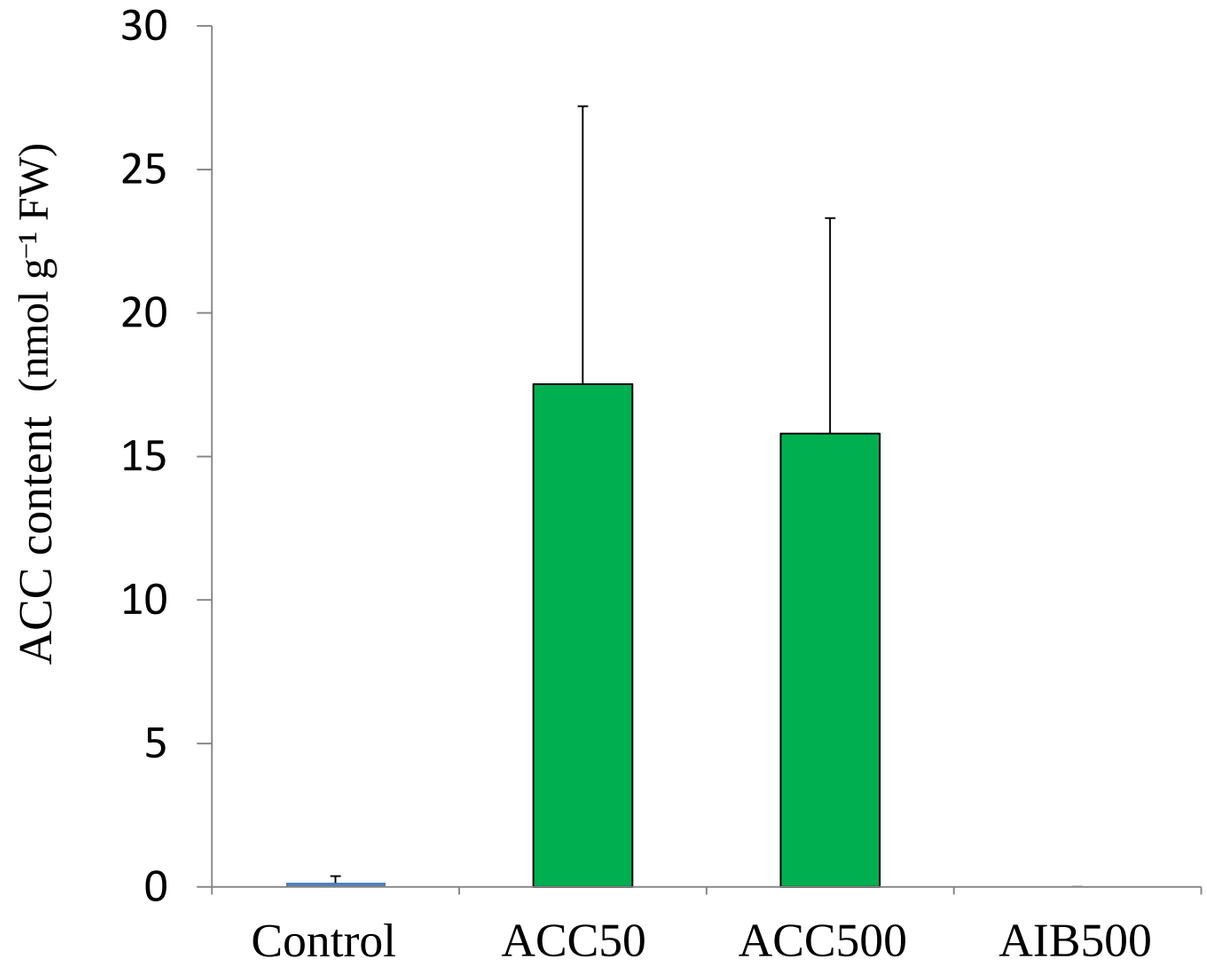
ACC content (nmol g⁻¹ FW)
30
25
20
15
10
5
0
Control
ACC50
ACC500
AIB500

Fig. 5

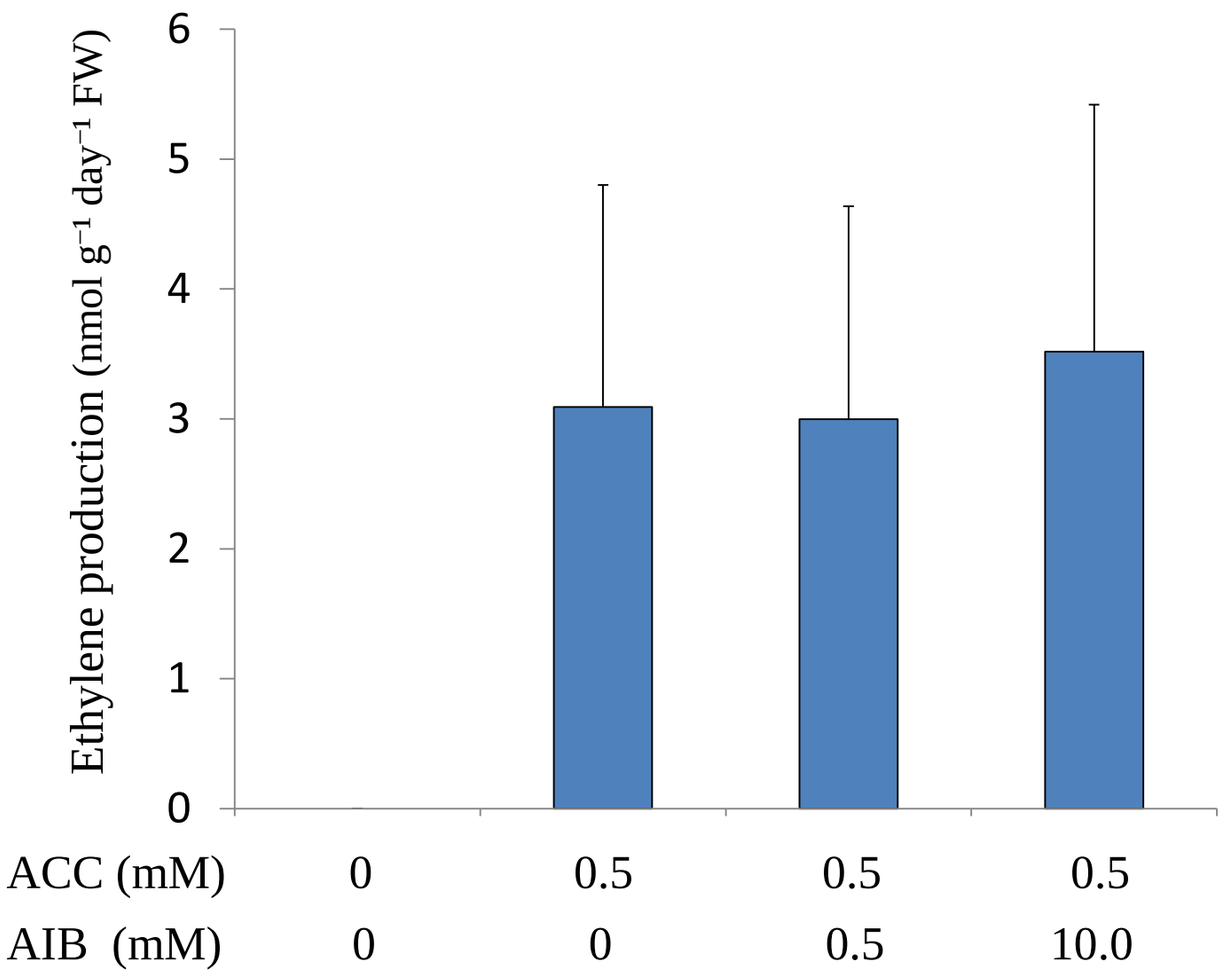
Ethylene production (nmol g⁻¹ day⁻¹ FW)
0
1
2
3
4
5
6
ACC (mM) 0 0.5 0.5 0.5
AIB (mM) 0 0 0.5 10.0

Fig. 6