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The role of coccoliths in protecting *Emiliania huxleyi* against stressful light and UV radiation

Running Title: Photoprotective role of coccoliths in *Emiliania huxleyi*

Juntian Xu[1,2], Lennart T Bach[3], Kai G Schulz[3], Wenyan Zhao[1], Kunshan Gao[1*], Ulf Riebesell[3]

[1]State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, Fujian, 361102 China;

[2] Key Laboratory of Marine Biotechnology of Jiangsu Province, Huaihai Institute of Technology, Lianyungang, Jiangsu, 222005 China;

[3]GEOMAR Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, Kiel, 24105 Germany

*Author for Correspondence: ksgao@xmu.edu.cn (Kunshan Gao)

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Abstract

Coccolithophores are a group of phytoplankton species which cover themselves with small scales (coccoliths) made of calcium carbonate ($CaCO_3$). The reason why coccolithophores form these calcite platelets has been a matter of debate since decades but has remained elusive so far. One hypothesis is that they serve a role in light/UV protection, especially in surface dwelling species like *Emiliania huxleyi* which can tolerate exceptionally high levels of solar radiation. In this study, we tested this hypothesis by culturing a calcifying and a non-calcifying strain under different light conditions with and without UV radiation. The coccoliths of *E. huxleyi* reduced the transmission of visible radiation (400-700 nm) by 7.5%, UV-A (315-400 nm) by 14.1% and UVB (280-315 nm) by 18.4%. Growth rates of the calcifying strain (PML B92/11) were about 2 times higher than those of the non-calcifying strain (CCMP 2090) under indoor constant light levels in the absence of UV radiation. When exposed to outdoor conditions (fluctuating sunlight with UV radiation), growth rates of calcified cells were almost 3.5 times higher compared to naked cells. Furthermore, relative electron transport rate was 114% higher and non-photochemical quenching (NPQ) 281% higher in the calcifying compared to the non-calcifying strain, implying higher energy transfer associated with higher NPQ in the presence of calcification. When exposed to natural solar radiation including UV radiation, maximal quantum yield of photosystem II was only slightly reduced in the calcifying but strongly reduced in the non-calcifying strain. Our results reveal an important role of coccoliths in mitigating light and UV stress in *E. huxleyi*.

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Key words: coccoliths, *Emiliania huxleyi*, light protection, growth, photosynthetic performance, UV radiation

1 Introduction

Coccolithophores are a group of marine phytoplankton species which are able to precipitate $CaCO_3$ in the form of small calcitic scales (coccoliths) surrounding the organic part of the cell. They contribute about by 1-10% to marine primary production (Poulton et al., 2007) and approximately 50% to pelagic deep ocean $CaCO_3$ sediments (Broecker and Clark, 2009). Blooms of coccolithophores can cover up to 8 million km^2 of the Earth's surface (Moore et al., 2012), and are considered to be important drivers of biogeochemical cycling (Rost and Riebesell, 2004).

Despite intense research on coccolithophore calcification and its biogeochemical relevance during the last decade, it is still an unresolved question why coccolithophores calcify (Young, 1994; Raven and Crawfurd, 2012). One hypothesis is that the layer of coccoliths surrounding the cell (coccosphere) protects the organism from excess light and UV radiation. This notion is supported by the exceptionally high light tolerance of the surface layer dwelling species *Emiliania huxleyi* (Nanninga and Tyrell, 1996; Gao et al., 2009).

Physiological studies investigating the light tolerance of *E. huxleyi* showed that the radiation wavelength matters in this context. The coccosphere does not seem to constitute a protection against very high intensities of photosynthetically active

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radiation (PAR) since non-calcifying *E. huxleyi* cells are equally resistant to photoinhibition as their calcifying counterparts (Nanninga and Tyrrell, 1996). This is in clear contrast to the influence of stressful ultraviolet radiation (UVR) on the cells where results from different physiological experiments support a protective role of the coccoliths (Gao et al., 2009; Guan and Gao, 2010; Gao et al., 2012). Protection from UVR or high light exposures by coccoliths may either work by physically shading intracellular organelles or by facilitating thermal dissipation through increased non-photochemical quenching (Xu and Gao, 2011). The underlying mechanisms, however, are not well understood and warrant further investigations.

In this study we explore in more detail how different PAR and UV radiation (280-400 nm) treatments affect calcifying and non-calcifying *E. huxleyi* cells. Specifically we address the question whether the coccosphere of *E. huxleyi* helps the cells to withstand stressful levels of PAR and/or UV radiation and whether calcification influences photochemical performance.

2. Materials and Methods

2.1 Materials and pre-culture conditions

Calcifying *E. huxleyi* (PML B92/11 isolated in the Raunefjord area, Bergen, Norway) and non-calcifying cells (CCMP 2090 isolated in the South Pacific) were used in the experiments. Both strains were grown in triplicate cultures (300 ml square glass bottles) at 15^{o}C in 0.2 µm filtered natural seawater (gathered from the Gulf of Biscay) at a photon flux density of 500 µmol photons m^{-2} s^{-1} on a 16/8 light/dark cycle.

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89 The natural seawater medium was enriched with 64 µmol L^{-1} nitrate, 4 µmol L^{-1}
90 phosphate, f/8 concentrations of a trace metal and vitamin mixture (Guillard & Ryther
91 1962), and 10 nmol kg^{-1} selenium. Pre-cultures and experimental incubations in
92 semi-continuously diluted batch cultures (>8 generations) ensured exponential growth
93 throughout the experiment.
94 2.2 Experimental setup
95 2.2.1 Indoor growth experiments
96 After pre-culture for at least 8 generations, the cells of calcifying and no-calcifying
97 strains were inoculated in the same glass bottles of 300 ml and cultured under the
98 same condition as pre-cultures, maintaining the cell concentrations at exponential
99 growth within a range of 3-10*10^4 cells/ml.
100 2.2.2 Outdoor growth experiments
101 Following the indoor growth experiment, the cells were transferred into quartz
102 tubes (100 ml) for the outdoor growth experiment and were exposed to natural solar
103 radiation at the institution's pier. The cultures were maintained outside in a
104 flow-through water tank, where the seawater temperature was maintained within a
105 range of 14-16^{o}C. After the cells had acclimated for 7 days under the solar radiation,
106 aliquots of the cell cultures were transferred to new quartz tubes filled with fresh
107 medium before measurements were taken. For the outdoor cultures, the cells received
108 60% full spectrum solar radiation (the quartz tubes wrapped with neutral density
109 screens). The daytime average intensities (from 7:00 am to 5:00 pm) of PAR, UV-A

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and UV-B which the cells received during the outdoor experiment were about 260 µmol photons m^{-2} s^{-1} (about 53 W m^{-2}), 12.4 and 0.34 W m^{-2}, respectively.

2.2.3 Short-term incubation experiments

Short-term incubation experiments were carried out to test UV effects around noon time on a cloudy day and sunny day, respectively. Three different radiation treatments were implemented as follows: 1) Cells in uncovered quartz tubes, receiving the full spectrum of solar radiation (above 280 nm, PAB treatment); 2) cells in quartz tubes covered with Folex 320 (Montagefolie, Nr. 10155099, Folex, Dreieich, Germany), exposed to UV-A and PAR (above 320 nm, PA treatment); and 3) cells receiving only PAR (P treatment) in quartz tubes covered with Ultraphan film 395 (UV Opak, Digefra, Munich, Germany). The transmission spectra of the quartz tubes and the cut-off foils are given by Zheng and Gao (2009). A time-course experiment was also conducted around noon under full solar spectrum conditions.

2.3 Absorptivity of coccoliths

We examined absorption spectra of the cells with or without coccoliths to get an indication on how much light and/or UV are blocked by the coccosphere. Therefore, calcified cells (Cal-C), de-calcified cells (Cal-R, see above) and cells of the naked strain (N-Cal) were filtered onto Whatman GF/F glass fiber filters (25 mm) which were subsequently placed at the window near the detector of a double beam UV-VIS-NIR spectrophotometer (PerkinElmer, Lambda950, USA). The absorption of the GF/F filter was corrected with a control filter which was soaked with particle free culture medium (Kishino et al., 1985).

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2.4 Growth measurement

Cell densities were measured during a period of 7 days with a particle counter (Coulter Z1, Beckman). The specific growth rate was calculated as: μ (d^{-1}) = (lnN_t-lnN_0)/t, where N_0 and N_t represent the cell concentrations at the beginning and the end of the incubations and t is the incubation time in days.

2.5 Chlorophyll fluorescence measurement

Parameters of in vivo induced chlorophyll a fluorescence of photosystem II were estimated by a phyto–pulse amplitude modulated fluorometer (Phyto-PAM, Walz). The maximum quantum yield of PSII (Fv/Fm) was calculated as: Fv/Fm=(Fm-Fo)/Fm; where Fo is the basal fluorescence under measuring light of 0.2 µmol photons m^{-2} s^{-1} and Fm the maximal fluorescence measured with a saturating light pulse of 5000 μmol photons m^{-2} s^{-1} (0.8 s) in dark-adapted (15 min) cells.

In order to compare the transmission of the same strain with or without coccoliths and to relate this to that of the non-calcifying strain, the calcified strain was de-calcified with HCl (1 mol/L, the final concentration is 0.01 mol/L) for 10 s and subsequent recovery of the pH with equimolar amounts of NaOH. Photochemical performance was measured for dark-adapted (15 min) cells in calcified, de-calcified or non-calcifying naked cells. De-calcified cells revealed Fv/Fm values similar to those obtained prior to de-calcification. The actinic light levels were set at 533, 1077 and 2130 μmol photons m^{-2} s^{-1}, respectively (growth light, saturated light and over-saturated light). Non-photochemical quenching (NPQ) was calculated as: NPQ = (F_m-F_m')/F_m', where F_m was the maximum fluorescence yield after dark adaptation and

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154 F_m' the maximum fluorescence yield under the actinic light levels.

155 To determine rapid light curves (RLCs, electron transport rate vs light), the cells
156 were exposed to 10 different PAR levels in sequence (87, 140, 263, 382, 449, 611, 778,
157 993, 1195 and 1391 μmol photons m^{-2} s^{-1}), each of which lasted for 20 s. The relative
158 electron transport rate (rETR) was assessed as: rETR = Yield × 0.5 × PFD, where the
159 yield represents the effective quantum yield of PSII (F_v'/F_m'); the coefficient 0.5 takes
160 into account that roughly 50% of all absorbed quanta reach PSII; and PFD is the
161 photon flux density of the actinic light (μmol m^{-2} s^{-1}) (Genty et al., 1989).

162 To examine immediate photochemical responses of the cells to UV radiation, the
163 cells were exposed to the three different solar radiations (see above) for 60 min during
164 noontime under natural solar radiation. The effective quantum yield was calculated as:
165 $F_v'/F_m' = (F_m' - Ft) / F_m'$, where F_m' and Ft are the maximal fluorescence and steady
166 state fluorescence in the light adapted cells, respectively.

167 2.6 Measurement of solar irradiances

168 Solar PAR was measured using a Quantum Scalar Laboratory Irradiance Sensor
169 (QSL-2100/ 2101, Biospherical Instruments, San Diego, USA). The measured values
170 were recorded every 10 s and saved on a computer. Solar UV-A and UV-B radiation
171 were measured with a radiometer (PMA 2100 Solar Light Co., Glenside, USA), the
172 mean irradiances of solar UV-A and UV-B during the experimental periods were
173 confirmed according to the ratios of UV-A /UV-B to PAR at the experimental location.

174 2.7 Statistics

175 The data were expressed as the means ± standard deviation (SD). Statistical

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176 significance of the data was tested with software of Origin 9.0 (one way ANOVA,
177 Tukey's post-hoc test). A confidence level of 95% was used in all analyses.
178
179 **3 Results**
180 The coccolith layer of *E. huxleyi* absorbed both visible and UV radiation. It reduced
181 the transmission of visible radiation (400-700 nm) by 7.5%, UV-A (315-400 nm) by
182 14.1% and UVB by 18.4% (280-315 nm) relative to decalcified cells and 6.5% for
183 PAR, 6.6% for UV-A and 5.1% for UV-B, relative to non-calcifying cells (Fig. 1). The
184 specific growth rate of calcifying *E. huxleyi* strain (PML B92/11) was about 2 times
185 higher than that of the non-calcifying strain (CCMP 2090) ($P < 0.05$) when grown at
186 500 μmol photons m^{-2} s^{-1} of PAR under indoor conditions (Fig. 2A). Growth rates of
187 both strains were significantly ($P < 0.05$) reduced when the cells were transferred
188 outdoor and exposed to natural solar radiation. However, under outdoor conditions,
189 growth rates of calcified cells were 3.5 times higher than those of the non-calcifying
190 cells, indicating that the latter was more harmed by the solar exposure than the former
191 (Fig. 2A). The cell diameter was not significantly different in the calcified cells
192 between the indoor and outdoor conditions ($P > 0.05$), but an 18% increase was found
193 in the non-calcifying cells after they had grown under the outdoor conditions for 7
194 days ($P < 0.05$) (Fig. 2B). The maximal quantum yield (Fv/Fm) decreased when the
195 cells were transferred from indoor to the outdoor conditions, reflecting a harmful
196 effect of solar radiation. The decrease of Fv/Fm, however, was much more
197 pronounced in the non-calcifying cells (27%) compared to calcifying cells (11%) (Fig.

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198 2C).

199 Calcified cells had significantly higher rETR, higher apparent light use efficiency
200 (α), and higher maximal electron transport rate ($rETR_{max}$), but significantly lower
201 light saturation parameters (Ik). The de-calcified cells of the calcifying strain showed
202 a remarkable decrease of rETRmax ($P < 0.05$), but did not show obvious changes in α
203 and Ik (Fig. 3, Table 1). Increased actinic light levels (acclimating light during the
204 fluorescence measurement) led to higher NPQ in both the calcifying and
205 non-calcifying strain (Fig. 4). Furthermore, calcified cells showed higher NPQ values
206 compared to non-calcifying cells ($p < 0.05$).

207 When exposed to full spectrum solar radiation, the quantum yield of calcified cells
208 showed no significant change during the first 30 min ($P > 0.05$). After 30 minutes,
209 quantum yield quickly dropped from about 0.35 to 0.22 for ~20 min ($P < 0.05$)
210 followed by a slight recovery in the last 25 minutes. A similar trend was observed in
211 the de-calcified cells with the key difference that the sharp decrease already happened
212 during the first 10 min. Quantum yield of the non-calcifying cells decreased
213 constantly for the first 50 minutes and remained at the low level thereafter (Fig. 5).

214 No effect of the radiation treatment (P, PA and PAB radiation) on the quantum yield
215 of calcified cells was observed after the cells grown under indoor condition were
216 transferred to outdoor solar radiation for 1h exposure (very cloudy day, average PAR,
217 UV-A and UV-B were 481µmol photons m^{-2} s^{-1}, 22.1 and 0.7 W m^{-2}, respectively) ($P >$
218 0.05). Quantum yield was significantly higher in the non-calcifying cells, however,
219 when they were exposed to UVA radiation (PA vs. P treatment, $P < 0.05$ Fig. 6A).

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Similar responses were observed when the same test was done on a sunny day with average PAR, UV-A and UV-B of 1605 µmol photons m^{-2} s^{-1}, 69 and 2.4 W m^{-2}, respectively. Here, the quantum yield of the calcified cells showed no significant difference between the different light treatments but it decreased significantly under PAB treatment compared to P treatments in the non-calcifying cells ($P < 0.05$) (Fig. 6B).

4 Discussion

Various hypotheses were proposed for the possible functions of coccoliths, but none of them is supported by sufficient evidence (Young, 1994; Raven and Crawfurd, 2012). One important function of coccoliths for surface-dwelling species such as *E. huxleyi* could be the protection against high photon flux densities, especially UV radiation (Berge, 1962; Young, 1994; Gao et al., 2009).

Some of our results support this hypothesis. The growth rate of the calcified cells of *E. huxleyi* grown under indoor conditions was about 2 times higher than that of naked cells. This difference came out even stronger, with growth rates 3.5 times higher in calcified versus naked cells, when the cells were exposed to full spectrum solar radiation (Fig. 2A). This could potentially be attributed to the screening of PAR, UV-A, and UV-B by coccoliths. Although the daytime PAR of solar radiation was reduced to about half of the light level of the indoor test, noon time PAR levels were higher than 500µmol photons m^{-2} s^{-1}, and the presence of UV could lead to more harms to the naked cells. Light protection by coccoliths is further supported by the

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Fv/Fm measurements. The maximum photochemical efficiency of PSII was only slightly reduced in calcified cells but significantly decreased in non-calcifying cells when they were exposed to natural solar PAR and UV radiation (Fig. 2C). Furthermore, photochemical performance of de-calcified cells decreased significantly faster and stronger with time compared calcified cells (Fig. 5).

The diameter of calcified cells did not significantly change when they were exposed to the full spectrum of solar radiation. The diameter of the non-calcifying cells, however, increased significantly (Fig. 2B). Perhaps, the non-calcifying cells experienced more DNA damage and so did not enter the S phase regularly (Buma et al., 2000). Alternatively, it may reflect a strategy to acclimatize to stressful solar UV radiation since it is well known that smaller cells are usually more sensitive to UV than their larger counterparts (Garcia-Pichel, 1994; Laurion and Vincent, 1998). Some field and laboratory studies showed increased cell size with increased UV exposures (Buma et al., 2000), which can be interpreted as adaptive or acclimation mechanism for protecting the cells against UV radiation. .

Several studies found that coccoliths do not protect *E. huxleyi* from excess PAR (Nanninga and Tyrrell, 1996; Houdan et al., 2005; Trimborn et al., 2007). However, UV radiation was not considered in these experiments. Our results showed that the non-calcifying cells were more sensitive to full spectrum solar radiation than calcified cells and even in the same strain, the photochemical performance of de-calcified cells decreased significantly when comparing the calcified cells. This suggests that coccoliths efficiently protect the cells from solar UV radiation.

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264 On the other hand, *E. huxleyi* appears to be more sensitive to UV-B irradiances than
265 other phytoplankton species, and its growth rate and physiological performances were
266 highly inhibited by UV radiation (Peletier et al., 1996; Buma et al., 2000; Xu et al.,
267 2011). However, competition tests for community changes are rare, and longer-term
268 experiments with less extreme UVR would be more ecologically and evolutionarily
269 relevant (Raven and Crawfurd, 2012). In our work, UVR had no significant effect on
270 the quantum yield of calcified cells regardless of high or low light condition but it
271 showed inhibition in non-calcifying cells when they were exposed to high solar light
272 (Fig. 6A, B). This provides further evidence for protection by coccoliths against UV
273 radiation.

274 On the cloudy day, no significant difference was observed among the treatments for
275 the calcifying cells; on the sunny day, under the fluctuating light (data not shown)
276 calcifying cells manage to refurbish damage to their photosynthetic apparatus by
277 balancing damage and repair (Gao et al., 2007). For the non-calcifying cells, on the
278 other hand, UV damage was not effectively repaired, leading to the observed negative
279 effect on photosynthetic performance.

280 In conclusion, the coccoliths of calcifying *E. huxleyi* play an important role in
281 protecting this species against harmful solar radiation especially UV-A and UV-B .
282 The reported absence of photoinhibition in this alga at high light levels is most likely
283 connected to the photoprotective role played by the coccosphere of *E. huxleyi*. With
284 shoaling of the upper mixed layer (UML) caused by global warming and progressive
285 ocean acidification, reduced thickness or the number of coccoliths (Gao et al., 2009;

286 De Bodt et al., 2010), cells of *E. huxleyi* living within the UML would be impacted
287 due to increased daily exposures to solar radiation.
288

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296

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Figure captions

Figure 1. Transmission spectra of cells with (Cal-C, calcifying strain) and without (Cal-R, calcifying strain with coccoliths removed artificially) coccolith cover and non-calcifying (N-Cal) cells of *Emiliania huxleyi*.

Figure 2. The specific growth rate (μ) (A), diameter (B) and maximum quantum yield

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(C) of PSII (Fv/Fm) of the calcified (Cal-C) and non-calcifying (N-Cal) cells of *E. huxleyi* grown in indoor and outdoor conditions. Different letters represent significant difference between the indoor and outdoor experiments. Different horizontal lines represent significant difference between the different strains.

Figure 3. The relative electron rate (rETR) of coccolith-covered (Cal-C), coccolith-removed (Cal-R) and non-calcifying (N-Cal) cells of *E. huxleyi* grown under indoor conditions as function of PAR. The cells had been grown for 12-22 generations under 500 μmol photons m^{-2} s^{-1} of PAR.

Figure 4. The non-photochemical quenching (NPQ) of coccolith-covered (Cal-C) and non-calcifying (N-Cal) cells of *E. huxleyi* grown under indoor conditions. Different letters represent significant difference among the light levels. Different horizontal lines represent significant difference among the different type cells.

Figure 5. The time course of quantum yield of coccolith-covered (Cal-C), coccolith-removed (Cal-R) and non-calcifying (N-Cal) cells of *E. huxleyi* under full spectrum solar radiation (noontime, average PAR, UV-A and UV-B were 1082µmol photons m^{-2} s^{-1}, 48.1 and 1.6 W m^{-2}, respectively).

Figure 6. The change of quantum yield of the calcified (Cal-C) and non-calcifying (N-Cal) cells of *E. huxleyi* when transferred from indoor to outdoor conditions, being exposed to PAR alone (P), PAR+UVA(PA) and PAR+UVA+B(PAB) for 60 min at around noon time. A, measured under a cloudy day (average PAR, UV-A and UV-B were 481µmol photons m^{-2} s^{-1}, 22.1 and 0.7 W m^{-2}, respectively); B, measured under

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a sunny day (average PAR, UV-A and UV-B were 1605 µmol photons m^{-2} s^{-1}, 69 and 2.4 W m^{-2}). Different letters represent significant difference among the light treatments. Different horizontal lines represent significant difference between the different strains.

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424 Table 1. Photosynthetic parameters of relative electron transport rate (Figure 3) as a
425 function of PAR, different letters represent significant difference (P<0.05) among the
426 treatments.

	α	$rETR_{max}$	I_k
Cal-C	0.23±0.02[a]	90.6±9.0[a]	1010.8±95.0[a]
Cal-R	0.20±0.01[a]	73.5±3.5[b]	986.3±27.4[a]
N-Cal	0.17±0.02[b]	42.3±8.5[c]	621.8±111.1[b]

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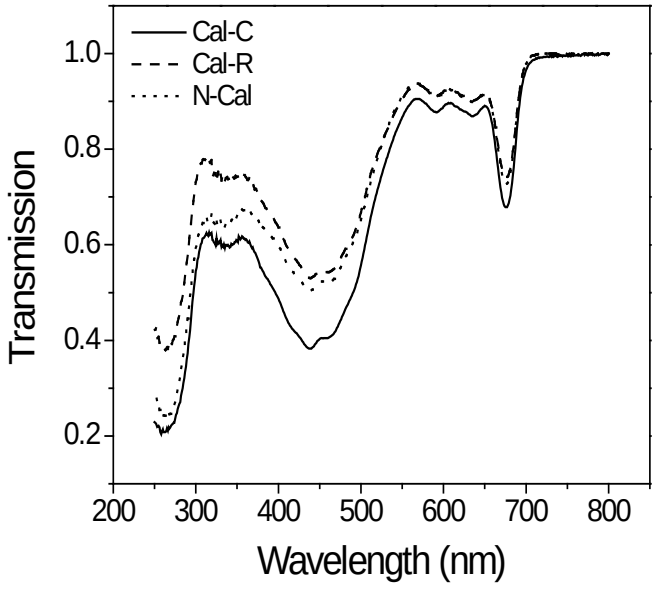

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431 Fig. 1

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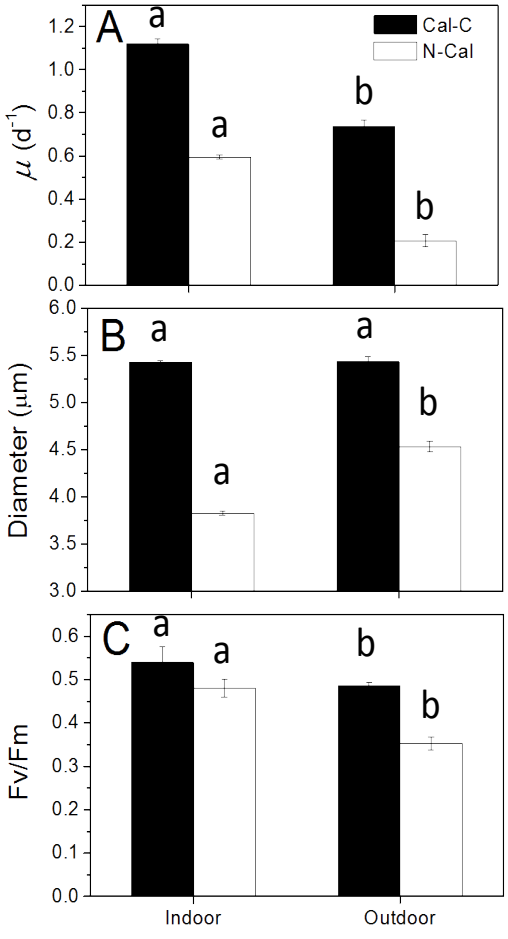

Fig. 2

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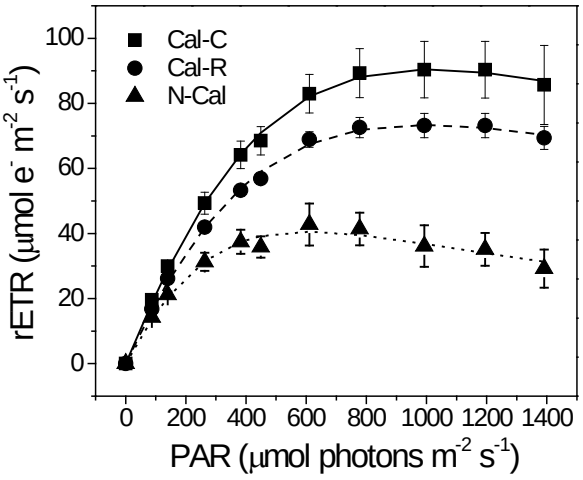

Fig. 3

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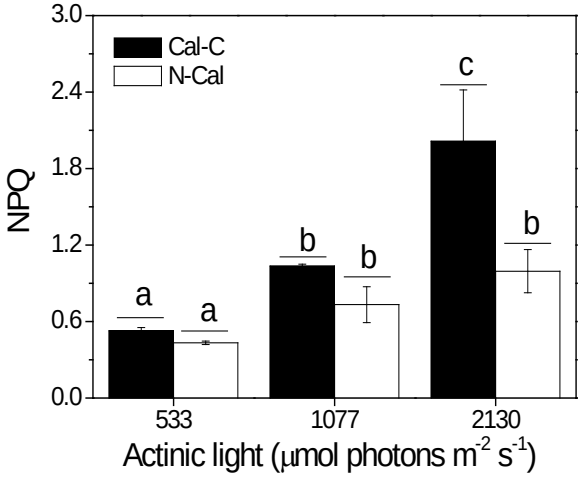

Fig. 4

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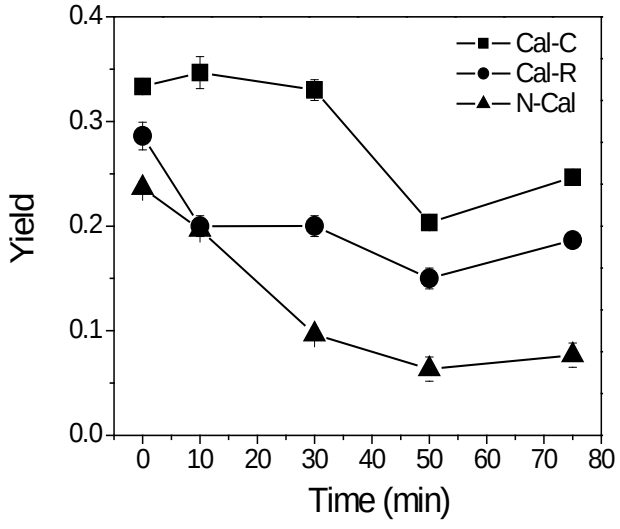

Fig. 5

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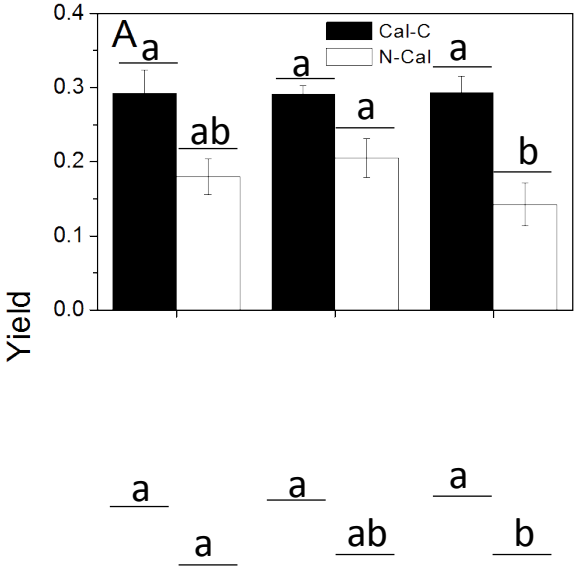

Fig. 6