

## Short communication

# Water depth-dependant photosynthetic and growth rates of *Gracilaria lemaneiformis*, with special reference to effects of solar UV radiation



Juntian Xu<sup>a,b,c</sup>, Xiaohui Zhang<sup>a</sup>, Qianqian Fu<sup>a</sup>, Guang Gao<sup>a</sup>, Kunshan Gao<sup>b,\*</sup>

<sup>a</sup> Jiangsu Key Laboratory of Marine Biotechnology, College of Marine Life and Fisheries, Huaihai Institute of Technology, Lianyungang, Jiangsu 222005, China

<sup>b</sup> State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, Fujian 361102, China

<sup>c</sup> Co-Innovation Center of Jiangsu Marine Bio-industry Technology, Lianyungang 222005, China

## ARTICLE INFO

## Keywords:

Depth  
*Gracilaria lemaneiformis*  
 Growth  
 Photosynthesis  
 Solar UV

## ABSTRACT

To optimize the farming strategy of economically important seaweeds, we grew *Gracilaria lemaneiformis* at different seawater depths under natural solar radiation with or without UV irradiances. Our results showed that solar UV radiation (UVR, 295–395 nm) significantly inhibited its growth and photosynthesis in the surface seawater; however, such a negative effects became inverse at 1.7 m with levels of UVR being about  $11.7 \text{ W m}^{-2}$ . The maximal growth rates were found at 1.7, 0.8 m when the thalli grown at PAR + UVR or PAR only conditions, respectively. UV-absorbing compounds in the algal thalli were up to 85% higher under PAR + UVR compared to PAR alone treatment and they increased with decreased depths even in the absence of UVR. The enhanced growth rate in the presence of UVR could be attributed to enhanced photosynthesis under moderate levels of solar radiation by UV-A. Our study clearly showed that UV-induced growth inhibition could be minimized or avoided by optimizing the alga's farming depth in different waters of different transparency, so that *Gracilaria's* biomass production could be increased.

## 1. Introduction

Solar UV radiation (280–400 nm), is the portion of the most biologically damaging solar spectrum that reaches the surface of the Earth (Häder et al., 2015). Solar UV-B (280–315 nm) varies spatio-temporally, being attenuated fast in water columns due to the properties of water and dissolved organic matters (Diaz et al., 2000; Wozniak and Dera, 2007). It was showed that photoactive depth of UV-B is as deep as 6.7 m in coastal water (Tedetti and Sempere, 2006). Most of marine primary producers in coastal area are distributed above this depth; therefore, they are naturally influenced by solar UV radiation (UVR 280–400 nm).

Solar UV radiation has been being recognized as one of environmental stressors to impact on marine macroalgae (Gao and Xu, 2010). It has been documented that UVR has negative effects on the growth, photosynthesis, DNA synthesis, key enzymatic activities, early developmental stage and spore germination of macroalgae (see the review by Häder et al., 2015 and references therein). Nevertheless, UV also showed positive effects on some macroalgae, with UV-A (315–400 nm) enhancing their growth (Henry and Van Alstyne, 2004; Xu and Gao, 2010a), photosynthesis (Xu and Gao, 2010b; Xu and Gao, 2016) and morphogenesis (Jiang et al., 2007) and aiding to photo-repair UV-B

induced DNA damage (Pakker et al., 2000). Additionally, UV-B (280–315 nm) could be involved in the recovery process of photoinhibition (Flores-Moya et al., 1999). Synthesis of UV-screening compounds, such as mycosporine-like amino acids (MAAs), phlorotannins and some unknown UV-B absorbing compounds, is the most efficient strategy for macroalgae to remedy harmful UV radiation (Gao and Xu, 2010).

*Gracilaria lemaneiformis* (Bory) Weber-van Bosse, an economically important red macroalga, was widely farmed in coastal waters in both northern and southern parts of China. Previously, we found that high levels of UV radiation decreased the growth and photosynthesis of *Gracilaria lemaneiformis*, however, low to moderate levels of UV-A enhanced its growth (Gao and Xu, 2008; Xu and Gao, 2010a). Growth of *Gracilaria lemaneiformis* could be dependent on growth depth that decides the daily doses of PAR and UVR (Xu and Gao, 2008). To further explore the roles of solar UV in regulating the growth of *G. lemaneiformis* and to develop optimal sea-farming strategy, we used a flow-through system to simulate the environmental conditions of *G. lemaneiformis* in farm area and studied the effects of solar UV and depth on the growth rate, photosynthesis and contents of UV-screening compounds of this alga.

\* Corresponding author.

E-mail address: [ksgao@xmu.edu.cn](mailto:ksgao@xmu.edu.cn) (K. Gao).

## 2. Materials and methods

### 2.1. Plant materials

*Gracilaria lemaneiformis* was collected from the farming area of Nan'ao island (117.1°E, 23.3°N), Shantou, China, and young thalli were selected and pre-cultured in tanks with flowing-through sand-filtered seawater before the experiments.

### 2.2. Experimental design

A flowing-through system was used for the experiment, which had been used previously for the macroalgal photosynthesis measurement (Gao and Xu, 2008). Quartz tubes (7 cm inner diameter and 40 cm long, about 1.5 L in volume) were used for the flow-through system with a seawater flow rate of 0.2 L min<sup>-1</sup>. About 3 g (fresh weight) thalli (6–9 individuals with the length of 20–30 cm) were fixed at stainless steel wires without shading and placed inside the tubes. To simulate the levels of solar radiation at different depths where *Gracilaria lemaneiformis* is grown, different layers of neutral density screens were applied to cut off the light intensities that the thalli received from 55% to 9%. The effects of solar UV were checked by covering the quartz tubes with different cutoff filters. Ultraphan 295 and 395 foils (UV Opak; Digefra, Munich, Germany) were used for PAB treatment (thalli received UVR and PAR) and P treatment (thalli received only PAR), respectively. Every treatment has three replications and there are 30 quartz tubes used in the experiment. The thalli were acclimated to different treatments for one week and then for the growth experiment.

### 2.3. Measurement of growth

The growth experiments were run for 8 days and fresh weight of the thalli was measured every two days. The relative growth rate (RGR) was estimated as follows:  $RGR = \ln(W_t / W_0) / t \times 100\%$ , where  $W_t$  is fresh weight of *G. lemaneiformis* after  $t$  days,  $W_0$  is the initial fresh weight.

### 2.4. Photosynthesis measurement

Photosynthetic O<sub>2</sub> evolution of *G. lemaneiformis* was measured in running water with a flow-through system according to Gao et al. (2012). The measurements were carried out at noontime on sunny days, during which neutral density screens were used to obtain the similar levels of daytime average light intensity that the thalli of *G. lemaneiformis* received during the growth periods. Other treatments were the same as the growth experiment. The rate of photosynthetic O<sub>2</sub> evolution ( $P$ ,  $\mu\text{mol O}_2 \text{ g FW}^{-1} \text{ h}^{-1}$ ) was obtained as follows:

$$P = (A - B) \times F \times 60 \times W^{-1},$$

where  $A$  and  $B$  were dissolved O<sub>2</sub> concentrations in the outlet and inlet, respectively;  $F$ , flow rate (L min<sup>-1</sup>);  $W$  is the fresh weight of thalli (g).

### 2.5. Determination of UV-absorbing compounds (UVACs)

About 0.1 g (fresh weight) thalli was extracted in 10 mL absolute methanol for 24 h at 4 °C in a refrigerator. The extract was centrifuged and then scanned from 250 nm to 750 nm with a UV spectrophotometer (UV 530, Beckman Coulter, Fullerton, CA, USA). The concentrations of UVACs were obtained according to Helbling et al. (2004).

### 2.6. Solar radiation measurements

Solar PAR and UV irradiances on the surface of seawater were continuously monitored with an ELDONET filter radiometer (Real Time Computer, Möhrendorf, Germany), which has three channels for photosynthetically active radiation (PAR, 400–700 nm), UV-A (UV-A,

315–400 nm) and UV-B radiation (UV-B, 280–315 nm). The irradiances were monitored every second and the mean over every minute was recorded in a computer (Häder et al., 1999). Levels of solar irradiances at different growth depths for *G. lemaneiformis* were simulated based on percentage of surface light and their underwater attenuation coefficients, which was measured by a diving ELDONET (Real Time Computer, Möhrendorf, Germany). Average daily solar doses in surface of seawater during the growth experiment were 10.98 and 1.61 MJ m<sup>-2</sup> for PAR and UVR, respectively. Solar PAR and UVR irradiances were up to 500 and 85 W m<sup>-2</sup> at the noontime, respectively.

### 2.7. Statistical analysis

The differences among the treatments and interaction between two factors were tested with two-way ANOVA, and the differences between P and PAB treatments under different light levels were tested with multivariate of variances (MANOVA, SPSS 18.0). A confidence level of 95% was used for all analyses.

## 3. Result

Average solar PAR irradiance the thalli received varied from 219 to 20 W m<sup>-2</sup>, which corresponded to the depths of 0–3.4 m. No significant differences of RGR were found between PAB (PAR + UVR) and PAR treatment in the thalli grown at low light level (2.4 and 3.4 m depths) ( $P > 0.05$ ), but with the increase of light intensity, the growth rate of *G. lemaneiformis* exposed to PAR only was lower than that of PAB treatment ( $P < 0.05$ ) when daytime mean solar UVR irradiance exceeded 12 W m<sup>-2</sup>. This trend was reversed at the solar UV threshold about 18 W m<sup>-2</sup> (about 1.7 m depth), and 10% and 16% decrease ( $P < 0.05$ ) for the thalli exposed to PAB condition were found compared with that of PAR treatment, with the solar UV irradiance were 21 and 39 W m<sup>-2</sup> (0.8 and 0 m depth), respectively (Fig. 1). There was significant interactive effect between depth and solar UV radiation ( $P < 0.05$ ).

The net photosynthetic rate showed similar pattern to that of the growth rate (Fig. 2). No significant differences between PAR and PAB treatments were found at low light intensities ( $P > 0.05$ ), but PAB induced higher net photosynthetic rate compared to PAR when solar UV increased to exceed 15.4 W m<sup>-2</sup> ( $P < 0.05$ ). Solar UV threshold for the reversion of net photosynthetic rate was  $\sim 23.4 \text{ W m}^{-2}$ , above which it declined with significant UV-induced inhibition. There was

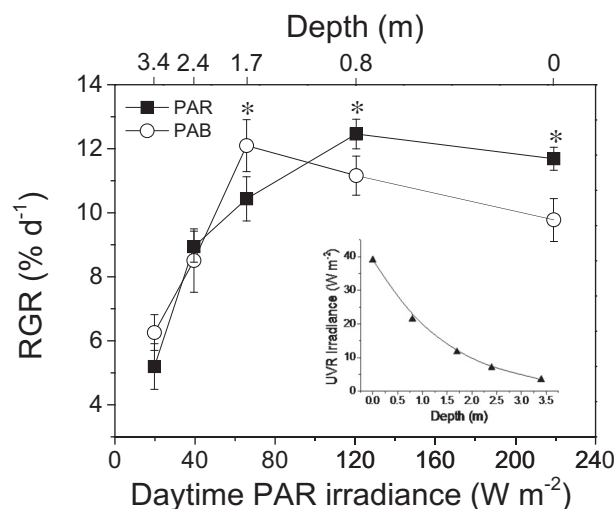


Fig. 1. The relative growth rate of *G. lemaneiformis* grew at solar PAR or PAR + UVR conditions as function of different simulated depths or daytime PAR irradiance. Inlet is the change of UVR irradiance with different simulated depths. Vertical bars represent  $\pm$  standard deviation of the means ( $n = 3$ ).

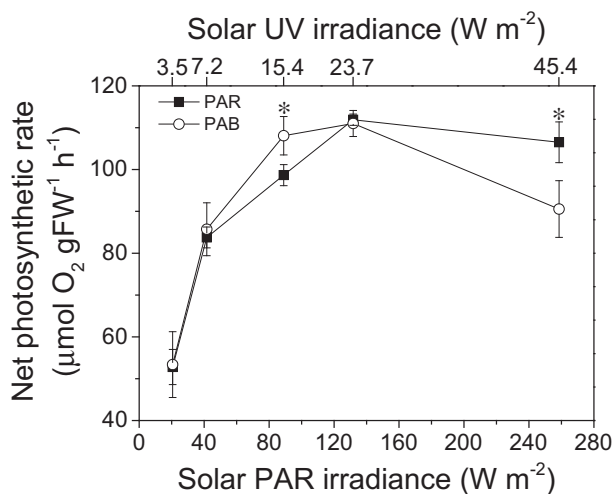


Fig. 2. Net photosynthetic rates of *G. lemaneiformis* as function of solar PAR and PAR + UVR. Vertical bars represent ± standard deviation of the means (n = 3).

significant interactive effect between depth and solar UV radiation (P < 0.05).

The contents of UV-absorbing compounds (UVACs) were significantly affected by daily solar dose (Fig. 3). The content of UVACs in *G. lemaneiformis* cultured at PAR only condition didn't significantly (P > 0.05) increase when solar PAR doses increased from 1 to 3.3 MJ m<sup>-2</sup>, but it increased (P < 0.05) by 51% and 218% when the daily PAR doses increased to 6 and 11 MJ m<sup>-2</sup>, respectively. For the thalli exposed to PAB treatment, there was a clear linear relationship between the content of UVACs and daily solar dose that *G. lemaneiformis* received (r<sup>2</sup> = 0.96, P = 0.0018), which showed 3 times higher contents of UVACs when the daily solar dose increased from 3.4 to 0 m (P < 0.05). There was no significant difference between PAR and PAB treatments at the depth of 3.4 m (P > 0.05), but solar UVR significantly increased the contents of UVACs at shallower depths (P < 0.05).

#### 4. Discussion

In the present study high solar UV had a significant inhibition effect on the growth of *G. lemaneiformis*, but this negative effect was reversed

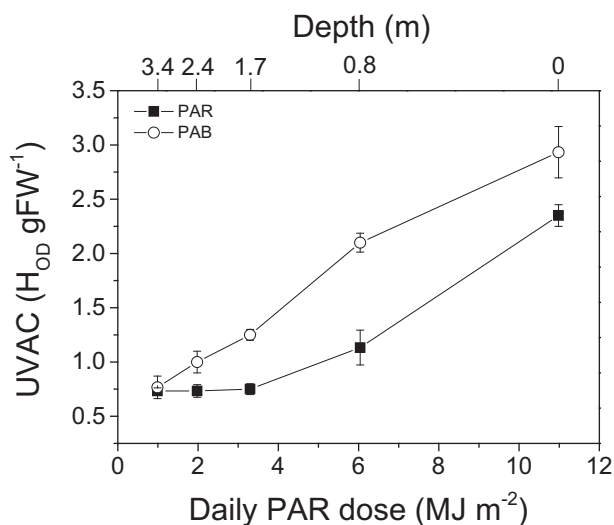


Fig. 3. The contents of UV-absorbing compounds (UVACs) grew at solar PAR or PAR + UVR conditions as function of different simulated depths or daily PAR dose. Vertical bars represent ± standard deviation of the means (n = 3).

with the increase of water depth. Although solar UV irradiances, especially UV-B, usually negatively impact growth of many macroalgae (Häder et al., 2015), moderate levels of UV-A can stimulate the growth of *G. lemaneiformis* (Xu and Gao, 2010a) and a brown macroalga *Fucus gardneri* (Henry and Van Alstyne, 2004). The intensity of UV radiation decreases with depth of water columns due to the absorbing and scattering properties of seawater (Wozniak and Dera, 2007). In the present work, UVR significantly enhanced the growth of *G. lemaneiformis* at the depth of 1.7 m, being optimal for its growth and deeper than PAR alone treatment, which was ~0.8 m. That is, growth-saturated level of solar radiation was about 120 W m<sup>-2</sup> for PAR only (Fig. 1) and it dropped to 66 W m<sup>-2</sup> in the presence of UVR. Such a difference could be attributed to the fact that UVR can play double edged effects on photosynthesis (Gao et al., 2007; Xu and Gao, 2010b). In the present study, without considering the depth-dependent performance, there was no significant difference for maximal growth rate between the two radiation treatments with or without UVR. In another word, the enhancement of growth rate by solar UV radiation at depth 1.7 m made up the growth difference between the thalli grown at this depth and 0.8 m. Excessive levels of solar PAR did not result in a significant decrease of growth rate for *G. lemaneiformis*, but higher solar UV radiation significantly inhibited it especially when the alga was grown at the surface of seawater. While energy of solar UV-A appears to be capable of driving or enhancing photosynthesis of *G. lemaneiformis* (Xu and Gao, 2010b; Xu and Gao, 2016), UV-B damages the photosystems especially in the present of visible light (Häder et al., 2015). However, since UV-B is attenuated faster than UV-A, at the optimal depth for growth, the alga could benefit more from UV-A and be less harmed by UV-B.

In macroalgae, synthesizing UV-absorbing compounds (UVACs) (Häder, 1997; Sinha et al., 2000; Carreto and Carignan, 2011; Hartmann et al., 2016) has been considered as the key strategy to cope with UVR. In *G. lemaneiformis*, the highest concentration of UVACs was observed in the thalli exposed to the highest UV radiation. Similar results were also reported in red macroalgae *Chondrus crispus* (Karsten et al., 1998), *Pyropia columbina* (Navarro et al., 2014) and a green macroalga *Ulva pertusa* (Han and Han, 2005). It has been shown that lower UVACs contents in another *Gracilaria* specie (*Gracilaria chilensis*) make it more susceptible to solar UV radiation (Gómez et al., 2005). In this work, we found that *G. lemaneiformis* can effectively protect the thalli against solar UV radiation by efficiently synthesize UVACs under high levels of solar radiation.

In sea-farming areas, *G. lemaneiformis* grew very well at the moderate depth, but its growth rate was significantly reduced when the thalli grown at the surface of seawater (Xu and Gao, 2008). This phenomenon could be attributed to the inhibitory effect of UVR in shallow seawater based on the findings of this study, though it accumulated higher contents of UVACs. To optimize primary production of macroalgae, such as *Gracilaria* plants, depth-dependent strategy should be considered in different locales.

#### Author contributions

J.X. contributed to carrying out the experiments. J.T. and K.G. contributed to experimental designs. J.X., X.Z. Q.F. and G.G. contributed to data analysis and paper writing.

#### Acknowledgements

This work was supported by the National Natural Science Foundation (Nos. 41476097 and 41106093), “333” project of Jiangsu Province, The Science and Technology Bureau of Lianyungang (SH1606) and Priority Academic Program Development of Jiangsu Higher Education Institutions.

## References

- Carreto, J.I., Carignan, M.O., 2011. Mycosporine-like amino acids: relevant secondary metabolites. Chemical and ecological aspects. *Mar. Drugs* 9, 387–446.
- Diaz, S.B., Morrow, J.H., Booth, C.R., 2000. UV physics and optics. In: De Mora, S., Demers, S., Vernet, M. (Eds.), *The Effects of UV Radiation in the Marine Environment*. Cambridge University Press, Cambridge, UK, pp. 35–71.
- Flores-Moya, A., Hanelt, D., Figueroa, F.L., Altamirano, M., Viñeola, B., Salles, S., 1999. Involvement of solar UV-B radiation in recovery of inhibited photosynthesis in the brown alga *Dictyota dichotoma* (Hudson) Lamouroux. *J. Photochem. Photobiol. B* 49, 129–135.
- Gao, K., Xu, J., 2008. Effects of solar UV radiation on diurnal photosynthetic performance and growth of *Gracilaria lemaneiformis* (Rhodophyta). *Eur. J. Phycol.* 43, 297–307.
- Gao, K., Xu, J., 2010. Ecological and physiological responses of macroalgae to solar and UV radiation. In: Israel, A., Einav, R., Seckbach, J. (Eds.), *Seaweeds and their Role in Globally Changing Environments*. Springer, Netherlands, pp. 183–198.
- Gao, K., Wu, Y., Li, G., Wu, H., Villafane, V.E., Helbling, E.W., 2007. Solar UV radiation drives CO<sub>2</sub> fixation in marine phytoplankton: a double-edged sword. *Plant Physiol.* 144, 54–59.
- Gao, K., Xu, J., Zheng, Y., Ke, C., 2012. Measurement of benthic photosynthesis and calcification in flowing-through seawater with stable carbonate chemistry. *Limnol. Oceanogr. Methods* 10, 555–559.
- Gómez, I., Figueroa, F.L., Huovinen, P., Ulloa, N., Morales, V., 2005. Photosynthesis of the red alga *Gracilaria chilensis* under natural solar radiation in an estuary in southern Chile. *Aquaculture* 244, 369–382.
- Häder, D.P., 1997. Penetration and effects of solar UV-B on phytoplankton and macroalgae. *Plant Ecol.* 128, 4–13.
- Häder, D.P., Lebert, M., Marangoni, R., Colombetti, G., 1999. ELDONET-European light dosimeter network hardware and software. *J. Photochem. Photobiol. B* 52, 51–58.
- Häder, D.P., Williamson, C.E., Wängberg, S.Å., Rautio, M., Rose, K.C., Gao, K., Helbling, E.W., Sinha, R.P., Worrest, R., 2015. Effects of UV radiation on aquatic ecosystems and interactions with other environmental factors. *Photochem. Photobiol. Sci.* 14, 108–126.
- Han, Y.S., Han, T., 2005. UV-B induction of UV-B protection in *Ulva pertusa* (Chlorophyta). *J. Phycol.* 41, 523–530.
- Hartmann, A., Holzinger, A., Ganzera, M., Karsten, U., 2016. Prasiolin, a new UV-sunscreen compound in the terrestrial green macroalga *Prasiola calophylla* (Carmichael ex Greville) Kützing (Trebouxiophyceae, Chlorophyta). *Planta* 243, 161–169.
- Helbling, E.W., Barbieri, E.S., Sinha, R.P., Villafañe, V.E., Häder, D.P., 2004. Dynamics of potentially protective compounds in Rhodophyta species from Patagonia (Argentina) exposed to solar radiation. *J. Photochem. Photobiol. B* 75, 63–71.
- Henry, B.E., Van Alstyne, K.L., 2004. Effects of UV radiation on growth and phlorotannins in *Fucus gardneri* (Phaeophyceae) juveniles and embryos. *J. Phycol.* 40, 527–533.
- Jiang, H., Gao, K., Helbling, E.W., 2007. Effects of solar UV radiation on germination of conchospores and morphogenesis of sporelings in *Porphyra haitanensis* (Rhodophyta). *Mar. Biol.* 151, 1751–1759.
- Karsten, U., Franklin, L.A., Lüning, K., Wiencke, C., 1998. Natural ultraviolet radiation and photosynthetically active radiation induce formation of mycosporine-like amino acids in the marine macroalga *Chondrus crispus* (Rhodophyta). *Planta* 205, 257–262.
- Navarro, N.P., Mansilla, A., Figueroa, F.L., Korbee, N., Jofre, J., Plastino, E., 2014. Short-term effects of solar UV radiation and NO<sub>3</sub>-supply on the accumulation of mycosporine-like amino acids in *Pyropia columbina* (Bangiales, Rhodophyta) under spring ozone depletion in the sub-Antarctic region, Chile. *Bot. Mar.* 57, 9–20.
- Pakker, H., Beekman, C.A.C., Breeman, A.M., 2000. Efficient photoreactivation of UVBR-induced DNA damage in the sublittoral macroalga *Rhodymenia pseudopalmata* (Rhodophyta). *Eur. J. Phycol.* 35, 109–114.
- Sinha, R.P., Klisch, M., Gröniger, A., Häder, D.P., 2000. Mycosporine-like amino acids in the marine red alga *Gracilaria cornea*-effects of UV and heat. *Environ. Exp. Bot.* 43, 33–43.
- Tedetti, M., Sempere, R., 2006. Penetration of ultraviolet radiation in the marine environment. A review. *Photochem. Photobiol.* 82, 389–397.
- Wozniak, B., Dera, J., 2007. *Light Absorption in Sea Water*. Springer, New York.
- Xu, J., Gao, K., 2008. Growth, pigments, UV-absorbing compounds and agar yield of the economic red seaweed *Gracilaria lemaneiformis* (Rhodophyta) grown at different depths in the coastal water of the South China Sea. *J. Appl. Phycol.* 20, 681–686.
- Xu, J., Gao, K., 2010a. UV-A enhanced growth and UV-B induced positive effects in the recovery of photochemical yield in *Gracilaria lemaneiformis* (Rhodophyta). *J. Photochem. Photobiol. B* 100, 117–122.
- Xu, J., Gao, K., 2010b. Use of UV-A energy for photosynthesis in the red macroalga *Gracilaria lemaneiformis*. *Photochem. Photobiol.* 86, 580–585.
- Xu, J., Gao, K., 2016. Photosynthetic contribution of UV-A to carbon fixation by macroalgae. *Phycologia* 55, 318–322.