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Control of Conchospores Formation in *Porphyra leucosticta*, a Genuine Short-Day Response

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ABSTRACT

The growth of *Porphyra leucosticta* collected from Alexandria shores, Egypt; was studied in culture under different controlled conditions, in order to precise the conditions for the formation and development of different types of gametes and spores produced by the different stages of this alga. The life cycle of *Porphyra leucosticta* contained two independent generations, a foliose thallus (Porphyra-stage) and a filamentous thallus (Conchocelis-stage). Sexual reproduction was observed. The formation of conchosporangia on the filamentous, Conchocelis-stage, proved to be a genuine short-day photoperiodic response, by night-break technique. Red light (620-660 nm) was found to be effective, night-break technique, in blocking the response. Phytochrome was proposed as a possible photoreceptor for such short-day photoperiodic response.

Key Words: *Porphyra*; Conchospores; Short-day; Mono-chromatic Light; life-cycle

INTRODUCTION

Our knowledge of life cycle of the foliose red alga *Porphyra* dates back to the middle of the 20th century (Drew, 1949, 1954). The development of some species of this alga was studied in culture by many authors (Tesng & Chang, 1955 a & b; Kornmann, 1961; Conway, 1964 a & b; Chen *et al.*, 1970; Ishimaru *et al.*, 2003). In the majority of studied species of *Porphyra,* the foliose thallus (*Porphyra*-stage) was found to alternate with a filamentous thallus (*Conchocelis*-stage). The morphological alternation was proven by many authors to be accompanied by a nuclear cycle, where the *Porphyra*-stage was haploid and the *Conchocelis*-stage was diploid (Magne, 1952; Giraud & Magne, 1968; Yabu, 1969, 1972; Yabu *et al.*, 1974; Choi *et al.*, 2002).

The objective of this work was to study the growth of *Porphyra leucosticte* collected from Alexandria shores, in Egypt, in culture under controlled conditions, in order to precise the conditions for the formation and growth of different types of gametes and spores produced by the different stages of this alga.

MATERIALS AND METHODS

Experimental alga. *Porphyra leucosticta* Thuret was collected in February 2003 from exposed sites in Abu-Qir and near Kayet-Bey citadel, Alexandria (Egypt). The alga was growing on rock surfaces in the littoral and supra-littoral zones. The collected alga had small size foliose fronds ranging from 4 to 5 cm in diameter, and were rose red in color (Fig. 1A); to coincide with the description given by Aleem (1993).

Experimental conditions. The collected foliose material was washed, by gentle shaking, in sterile medium then maintained in 250 mL Erlenmeyer flasks containing 100 mL enriched sea-water (ES) medium (Provasoli, 1968), and kept at 10 ± 1ºC, under daily light/dark cycles of 16h/8h (light with an irradiance of 20 ìmol m^{-2} s^{-1} fluorescent tubes lights). Experiments at the foliose-stage of alga were carried out in the Erlenmeyer flasks, while those on the filamentous-stage were carried out in small tubes (30 x 40 mm) with plastic caps containing 15 mL ES medium. The medium was changed weekly. Experiments were carried out in ventilated incubators (built by the author, based on design proposed by Professor F. Magne, Univ. Pierre and Marie Curie, Paris, France, pers. com.), providing controlled conditions of temperature (10, 15 and 20±1ºC) and light intensity. Photoperiod was regulated by micro-flash timer to provide the 16h/8h (long-day; LD) conditions, 12h/12h (medium-day; MD) and 8h/16h (short-day; SD). For this purpose, the flasks or tubes were manually transferred to dark-boxes kept inside the incubators.

In monochromatic light experiments, light was provided by halogen bulbs (Osram) combined with 15 filters (Type "NBIF", Oriel Corp., USA) of wavelengths nearly covering the visible spectrum (permitting wave lengths: 400, 420, 450, 480, 500, 520, 550, 580, 600, 620, 640, 660, 680, 700 and 750 nm, each with a half band width ± 10 nm). Irradiance was approximately 0.3 µmol m^{-2} s^{-1}. Materials were examined daily or after 2-3 weeks, according to the experimental protocol, *in vivo* with a binocular dissecting microscope.

In order to study the reproduction of the foliose-stage, the foliose blades in stock were divided into small squares (0.5 x 0.5 cm), 10 of these squares were placed in each of 9 flasks (containing 100 mL ES medium), three flasks were placed in three incubators adjusted at 10, 15 and 20 ± 1ºC, each with three photoperiods LD, MD and SD and the above-mentioned irradiance for all conditions (20 µmol m^{-2} s^{-1}).

The filamentous-stage produced in the first experiment was separated in a flask, divided into nine groups in small plastic caped tubes which were distributed in three incubators having the above mentioned conditions.

In a third experiment the filamentous-stage was separated into 16 groups in small plastic caped tubes (containing 15 mL ES medium) kept at 15±1ºC (always at the same irradiance) and a photoperiod of SD (8h light / 16h dark) where the dark period was interrupted with 1 hour night break by either white light (irradiance 20 µmol m^{-2} $s^{-1)}$ or one of 15 monochromatic light (irradiance 0.3 µmol m^{-2} s^{-1}) mentioned above.

RESULTS

The obtained results for the effect of temperature and photoperiod conditions on the reproduction of the foliose-stage (*Porphyra*-stage), showed that monospores (aplanospores) and young foliose thalli (Fig. 1F) were produced from the initial squares at all temperatures and under all photoperiods used. Their production was higher under LD and at 20ºC than other conditions. Proliferations of young foliose thalli were observed (Fig. 1G) on many of the initial squares at 15ºC and under LD conditions. At 20ºC and under LD the filamentous-stage (*Conchocelis*-stage) was produced in relatively large quantities (Fig. 2D). Squares under the latter conditions were thoroughly examined, where spermatangial patches were clearly observed in certain squares (Fig. 2A); carpogonia (Fig. 2B) and dividing carposporangia (Fig. 2C) were only observed in vertical sections.

Results concerning the effect of temperature and photoperiod conditions on the reproduction of the filamentous-stage (*Conchocelis*-stage) revealed that monospores (aplanospores) were produced in sufficient

Fig. 1. *Porphyra leucosticta* foliose thallus collected from Alexandria shores
Habit of foliose thallus (A); surface view of vegetative cells (B); monospores liberated from the squares of the foliose thallus (C); details of a monospore (D); germination of a monospore (E); young foliose thallus (F); proliferations of young foliose thalli from square margin (G).

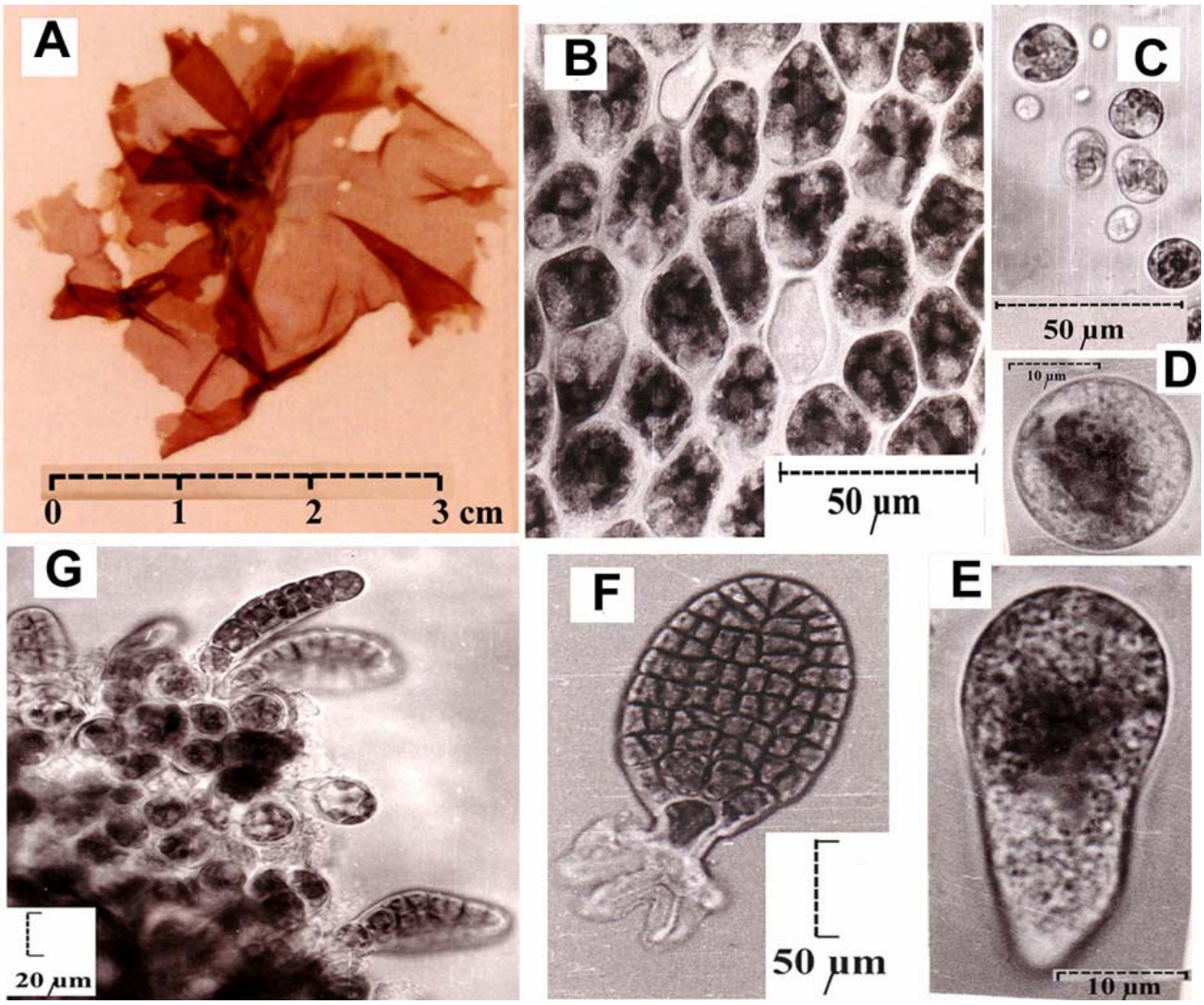
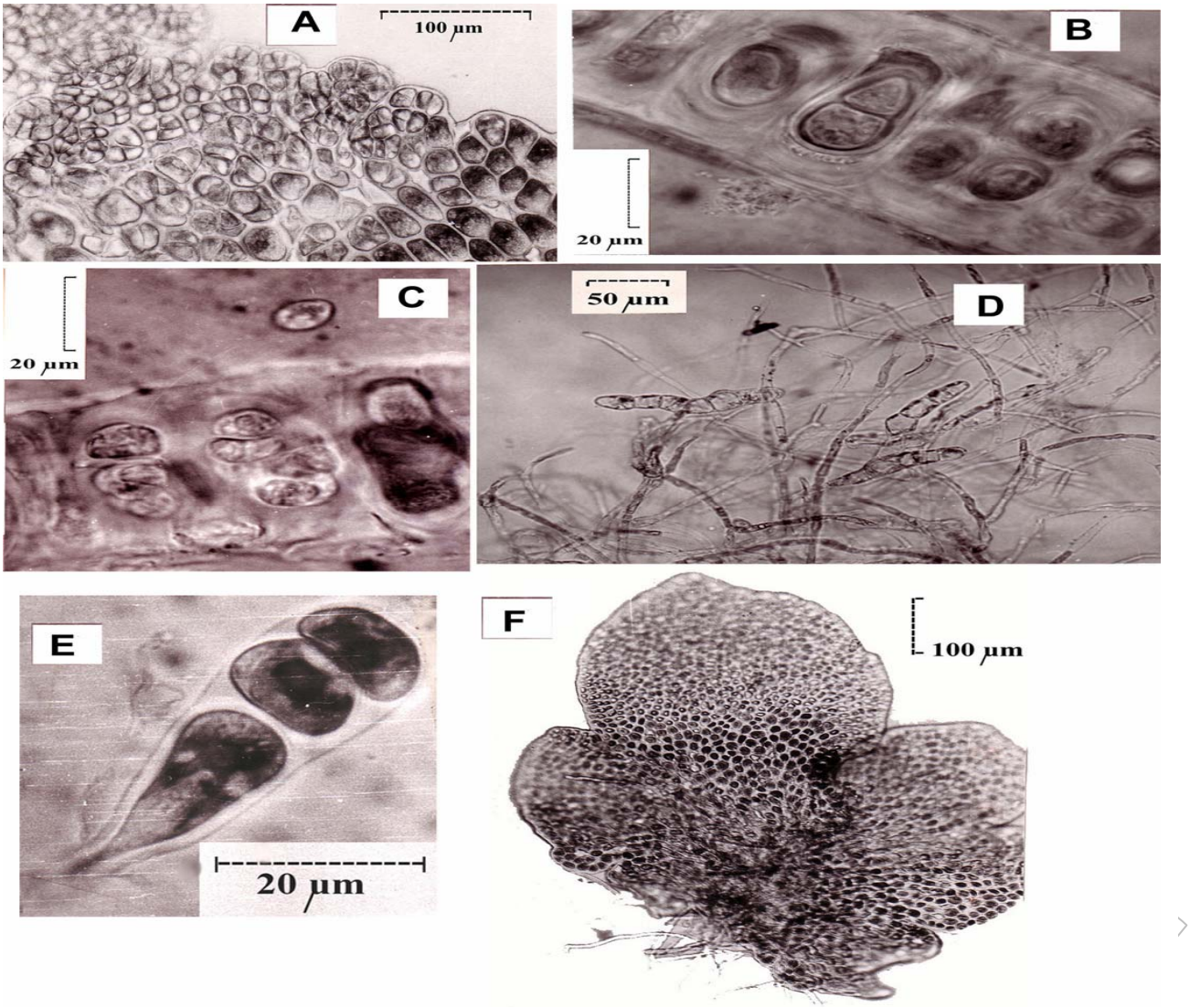


Fig. 2. *Porphyra leucosticta* foliose and filamentous stages
Patches of spermatangia (A); V.S. in the sexual foliose thallus showing dividing carpogonium (B) and carposporangium liberating a carpospore (C); filamentous thallus showing conchosporangia (D); stage of the germination of a conchospore (E); young foliose thallus originated from a conchospore (F).



quantities under MD and LD photoperiods at 15 and 20ºC. At lower temperature, 10ºC under SD conditions, production of conchosporangia was observed (Fig. 2D). The filamentous thalli carrying conchosporangia were transferred to different temperatures and photoperiods. Conchospores germinated to young foliose thalli (Fig. 2 E & F) only at 10 and 15ºC under MD and LD conditions.

In the previous experiment conchosporangia were produced under SD photoperiod conditions (10ºC). An experiment was conducted using night-break technique to examine whether conchosporangia formation was a genuine SD response? The results obtained indicated that the formation of conchosporangia was inhibited by 1 h night break. Using monochromatic light of very low irradiance level, instead of white light, the production of conchosporangia was completely inhibited with night-breaks at wave lengths 620, 640 and 660 nm, other wave lengths used, however, were not effective.

DISCUSSION

The results obtained in this work indicated that the life cycle of *Porphyra leucosticta* collected from Alexandria (Egypt) shores contained two independent generations, a foliose thallus (*Porphyra*-stage) and a filamentous thallus (*Conchocelis*-stage). These results coincide with Kornmann (1961) observations on the Atlantic form of this species and those reported by many authors on other *Porphyra* species (Tseng & Chang, 1954, 1955; Hawkes, 1978; Cole & Conway, 1980; Ishimaru *et al.*, 2003). The foliose thallus reproduced asexually by monospores (aplanospores), in all

the experimental conditioned used. The formation of young foliose proliferations from squares under certain conditions (LD, 15ºC), might be attributed to failure in the liberation of monospores and their germination *in situ*. Asexual reproduction of the foliose thallus of *Porphyra* spp. is common in the Pacific Ocean and was observed in four of the six species of economic importance in Japan (Kurogi, 1972; Miura, 1975; Mizuta *et al.*, 2003).

The formation of *Conchocelis*-stage under LD (20 ºC) conditions showed the occurrence of sexual reproduction. Examination of the starting squares of the foliose thallus at this stage confirmed the presence of sexual organs for this species. According to Aleem (1993) and our personal observations, the foliose thallus of *P. leucosticta* was ordinarily found in winter and spring. So, it is not surprising that sexual reproduction and the formation of *Conchocelis*-stage occurred under LD at 20ºC conditions, resembling summer season under natural conditions in Alexandria. This appeared to maintain the perpetuation of this species in nature at different stages.

The filamentous *Conchocelis*-stage of this species produced conchosporangia under short-day conditions (at 10-15ºC). This response proved to be genuine by using the night-break technique. These results are similar to those obtained in many species of *Porphyra* (Kurogi, 1959; Kurogi & Sato, 1962; Dring, 1967 a & b; Rentscher, 1967; Notoya *et al.*, 1999). Genuine short-day photoperiodic responses were recorded in other genera of red algae (Abdel-Rahman, 1982 a & b).

Study on the action spectrum of monochromatic light as night-breaks for the formation of conchosporangia indicated the efficiency of red light (620-660 nm) in blocking the response. Therefore, Phytochrome might be the photoreceptor for such response, as in higher plants (Vince-Prue, 1975). Further investigations are however needed to confirm these findings.

Finally, the data suggested that the life cycle of *Porphyra leucosticta* is controlled in nature by certain timing mechanism; the foliose-stage exists in winter and spring while the filamentous-stage might exist in summer and autumn. The latter stage had never been announced or collected from Alexandria (Egypt), and is awaited to be found especially inside animal shells, where this stage of other species of *Porphyra* was usually collected.

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