INFLUENCE OF IRRADIANCE AND SALINITY ON THE GROWTH OF CARPOSPORELINGS AND JUVENILE TETRASPOROPHYTES IN *GRACILARIA GRACILIS* (STACKHOUSE) M. STEENTOFT, L.M. IRVINE & W.F. FARNHAM (RHODOPHYTA; RHODOPHYCEAE; GRACILARIALES) AND AGAR YIELD

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ملخص

تأثير الإضاءة والملوحة على نمو الأبواغ الثمرية الصغيرة والأطوار البوغية الرباعية الناشئة لدى الطحلب الأحمر غراسيلاريا غراسيليس :اهتمت هذه الدراسة بالأبواغ الثمرية والنباتات الصغيرة الناتجة عنها والمتمثلة في الطور رباعي الآبواغ للطحلب الأحمر غراسيلاريا غراسيليس الموجودة في بحيرة بنزرت. وتمت متابعة النمو ومردود الأغرة تحت تأثير ثلاثة مقادير معينة من شدة الإضاءة والدورة الضوئية والملوحة.وقد بينت النتائج أن أفضل نمو الأبواغ الثمرية تم تسجيله تحت شدة إضاءة 60 ميكرو مول فوطون/م^2/ث ودورة ضوئية ب16:8 س ودرجة ملوحة 40غ/ل(260.00±60.45مكرومتر) وذلك بعد لربعة أسابيع من الاستزراع. أما أعلى نسبة لبروز"الأوراق" والتي بلغت 100٪ فقد تم الحصول عليها تحت شدة إضاءة 32 ميكرو مول فوطون/م^2/ث ودورة ضوئية 8:16س وملوحة تقدر ب30غ/ل. أما أكثر وزن للنباتات الصغيرة الناتجة عن نمو الأبواغ الثمرية وهو الطور رباعي الأبواغ فقد بلغ حوالي 6000مغ بعد 228 يوما من الاستزراع عند استعمال درجة ملوحة تقدر ب40غ/ل. وأما أطول حجم لها فقد بلغ16.25± 0.36 صم باستعمال نفس الملوحة.و أما أقصى مرود الأغرة (30.75±7.5 ٪) فتم تسجيله باستعمال أدنى مقادير الإضاءة والملوحة التي وقع اختبارها في هذه الدراسة. وقد بينت النتائج المتحصل عليها إمكانية استزراع غراسيلاريا بالأبواغ للحصول على طحالب بالغة ومن ثم استخراج منها عدة مواد هامة كالأغرة.

كلمات مفاتيح : طحلب أحمر-غراسيلأريا غراسيليس- ابواغ ثمرية- طور رباعي الأبواغ- نمو-أغرة

RESUME

Influence de la lumière et la salinité sur la croissance des petites carpospores et des jeunes tétrasporophytes chez *Gracilaria gracilis* (Stackhouse) m. Steentoft, L.m. Irvine & W.F. Farnham (Rhodophyta; Rhodophyceae; Gracilariales) et sur le rendement en agar-agar : Cette étude a été réalisée sur les carpospores et les petites plantules (tétrasporophytes juvéniles) qui en sont issues de la rhodophycée *Gracilaria gracilis* de la lagune de Bizerte. Elle a pour objectif de tester l'effet de trois intensités lumineuses, photopériodes et salinités sur la croissance et le rendement en agar-agar. Les résultats montrent que la croissance la plus importante des carpospores a été enregistrée sous une intensité lumineuse de 60 µ mol photons m-^{2}s^{-1}, une photopériode de 8 :16 h et une salinité de 40 psu (260 ± 64.48 µm) après quatre semaines de culture. Le pourcentage le plus élevé (100 %) des frondes érigées a été obtenu en fin de culture sous 32 µ mol photons m-^{2}s^{-1}, une photopériode 16 : 8 h et une salinité de 30 psu. Le poids le plus élevé (environ 6000 mg) des petites plantules issues des carpospores a été enregistré après 228 jours de culture à 40 psu. La longueur maximale (16.25 ± 0.36 cm) a également été obtenue à une salinité de 40 psu. Le rendement en agar-agar a atteint 30.75 ±7.15 % du poids sec de l'algue avec les valeurs les plus faibles d'intensité lumineuse, de photopériode et de salinité. Cette étude a mis en évidence la possibilité de culture de *Gracilaria* à partir des spores afin d'obtenir des plantes adultes et d'en extraire par la suite des substances d'intérêt tel que l'agar-agar.

Mots clés: *Gracilaria gracilis*; croissance ; carpospores; tetrasporophytes; radiation; salinité; agar

ABSTRACT

The present study aims to investigate the effects of irradiance and salinity on the growth and agar yield in early stages of development of carposporelings and young tetrasporophytes of *Gracilaria gracilis* in the laboratory conditions. Three levels of irradiance, photoperiod and salinity were tested. The results showed that the highest growth in basal disc of carposporelings was recorded at 8:16h light/dark, 60 µmol photons m^{-2}s^{-1}, and a salinity of 40 psu after four weeks of culture. The optimal percentage of erect fronds occurred at 16:8 h light/dark, 32 µmol photons.m^{-2} s^{-1} and a salinity of 30 psu .The highest weight (about 6000mg) of juvenile tetrasporophytes was recorded at 40psu after 228 culture days. The maximum of length was also obtained at 40 psu at the end of the study. The best agar yield was recorded at 8:16 h light/ dark and a salinity of 20 psu. This study proved that *Gracilaria* may be cultivated using spores in order to obtain mature plants and then extract useful compounds such as agar.

Key words: *Gracilaria gracilis*; growth; carposporelings; tetrasporophytes; irradiance; salinity; agar

INTRODUCTION

The demand for seaweeds of economic interest is in constant rise throughout the world. Several species are exploited in several countries such as China, Japan, Philippines, Argentina and Chile. The most cultivated species belonging to the Chlorophyta are *Enteromorpha*, *Monostroma* and *Caulerpa lentillifera* (Pérez, 1997), the Pheophyta are *Laminaria*, *Macrocystis* and *Undaria* (F.A.O., 2003), the Rhodophyta are *Gelidium*, *Gracilaria*, *Eucheuma*, *Kappaphycus*, and *Hypnea* (Dawes, 1998; Freile-Pelegrin and Robledo, 2006; Phang, 2000). These species are farmed either for food or phycocolloids (agar,carrageens and alginates). Among the red seaweeds, the genus *Gracilaria* and *Gelidium* have a considerable importance as an agarophyte and is the principal source of raw material to the agar industry worldwide (Zemke-White and Ohno, 1999; Smit, 2004). The agar industries consume 72300 dry tons of agarophytes annually and produce approximately 9600 tons agar with a value of US$ 173 million of which *Gracilaria* alone accounts for about US$138 million (Bixler and Porse, 2010). The cultivation of different species of *Gracilaria* including *G. verrucosa* (Hudson) Papenfuss 1950 (Padhi et *al*.2011) and *G. dura* (C.Agardh) J.Agardh 1842 (Gupta et *al*.2011) has been carried out in several countries. The worldwide increasing demand for agar resulted in an over exploitation of wild stocks leading to the development of viable cultivation methods for their large-scale cultivation in the sea (Critchley, 1993).The feasibility of field cultivation of *Gracilaria dura* using both vegetative fragments and carpospores has also been successfully demonstrated (Mantri et *al*. 2009).Indeed, two methods of culture were followed: the vegetative way and by spores. The latter technique is applied in order to overcome the lack of raw material and to ovoid the overexploitation of natural stocks. In this case, the culture in the laboratory is an alternative approach to have plantlets able to cultivate either in nature (sea or lagoon), or in tanks, ponds and artificial basins. Different trials have been performed on *Gracilaria* in many countries such as Chile (Edding et *al*. 1987), and India (Jayasankar and Varghese, 2002; Jayasankar et *al*.2005). In China, the foremost country in farming of seaweeds.different species are cultivated either by vegetative method, like *Gracilaria parvispora* Abbott, I.A. (1985) or spores like *G. tenuistipitata* var. *liui* Zhang and Xia (1988) and *G. gigas* Harvey, W.H. (1860) (FAO, 2009). Nevertheless, many other countries belonging to Europe (Tasende and Fraga, 1997), America (Santelices and Doty, 1989) and Africa (Mshigeni and Mziray, 1979) have also become interested in studying some seaweeds, both for exploitation and farming. Among these seaweeds, *Gracilaria gracilis* (Stackhouse) M. Steentoft, L.M. Irvine et W.F. Farnham, previously known as *Gracilaria verrucosa* (Hudson) Papenfuss is considered as a good source of raw material for agar production in some countries in South Africa, namely in Namibia where it is wildly met. In this context, many culture trials have been successfully carried out (Molloy and Bolton, 1996; Robello et *al*. 1996; Jaffray et *al*. 1997). The seaweed culture success requires controlling many factors, namely the environmental ones. The genus *Gracilaria* has been the object of several studies around the world in order to assess the growth and the variation of different products with respect to several abiotic factors (Edelstein et *al*. 1976; Daugerthy and Bird 1988; Santelices and Doty 1989; Wilson and Critchley, 1997; Floreto, 1998; Glenn et *al*. 1998; Mollet et *al*. 1998; Israel et *al*.1999; Sanchez et *al*. 2000; Ramlov et *al*. 2012).

In Tunisia, the interest in studying seaweeds and their potential and valorization has started as early as 1996-1997. *Gracilaria* was investigated along Tunisian coasts, namely in Tunis and Bizerte lagoons. The biomass and the cartography were established (Ksouri et *al*.1997).Moreover, agar yield and quality fluctuation in *Gracilaria gracilis* of Bizerte lagoon was recently studied with respect to some hydro biological parameters (Ben Said *et al*.2015). Besides, many experiments were conducted to cultivate *Gracilaria* in Bizerte lagoon by vegetative way (Ksouri et *al*.1999; Mensi et *al*.2010). At present, there are no industrial companies of phycocolloids in Tunisia and the estimated biomass of *Gracilaria* in 1997 has been about 4700 tons of wet raw material in Bizerte lagoon is too limited to support a viable industry. That's why different experiments were conducted to increase the biomass of *Gracilaria* following artificial cultivation. In order to have some autonomy in the future, several trials have been carried out in the laboratory to study the release of spores and their culture in *Gracilaria* (Ben Saïd *et al*.2011; Ben Saïd et Aouini, 2014)). The present study aims to obtain carposporelings from *Gracilaria gracilis* (Stackhouse) M. Steentoft, L.M. Irvine et W.F. Farnham 1995 and then cultivate them in the laboratory under two main environmental factors (light and salinity) to have juvenile tetrasporophytes suitable for transplantation in the sea. This step requires some scientific and technical knowledge in order to ascertain the optimal conditions both for cultivation and agar yield.

MATERIAL AND METHODS

Algae sampling and carpospore induction

The red alga *Gracilaria gracilis* was collected from Bizerte Lagoon (37°8'-37° 14 'N; 9°48'-9°56' E) in November 2012. Samples were placed in plastic bags and brought to the laboratory, where they were brushed, rinsed with sterilized and filtered seawater to remove sand, epiphytes etc. Female plants are easily recognized by their cystocarps. After cleaning female plants bearing cystocarps, the algae were weighed. A sample of 3 g was placed in darkness in wet paper for 2 hours. Following the dark treatment, the algae were placed in100 mm diameter glass Petri dishes with approximately 20 ml of filtered seawater. They were then kept under 80 µmol photons.$m^{-2}s^{-1}$ irradiance and 12: 12 h light: dark cycle for 24 h at 16°C. Irradiance was provided by OSRAM L18 W/10 daylight white fluorescent lamps (Germany). Experiments were conducted in ten replicates in order to collect the maximum of carpospores.

Culture medium

After the release of carpospores, two steps of cultivation were carried out in SWM3 culture medium (Chen and Ren 1983), but modified. The components of the final medium are:$NaNO_3$(170 $mg.l^{-1}$);NaH_2PO_4(15.6 $mg.l^{-1}$); EDTA (11.16 $mg.l^{-1}$); $FeCl_3$ (0.126 $mg.l^{-1}$) and Tris(500 $mg.l^{-1}$).Three salinities were tested:20,30 and40psu. The two first concentrations were obtained following seawater dilution with distilled water[1]. The components described above were added to each seawater solution and then autoclaved. The replenishment of the culture medium was made weekly.

****Assessment of basal disc development and percentage of erect fronds***

After the carpospores release, they were replaced in new Petri dishes containing the same culture medium. Two glass slides were placed in the Petri dishes to allow the settlement of carpospores and subsequently control growth under a photonic microscope (Nikon, type 104). Cultures were conducted under three combined factors: Photon Flux Densities (PFD) of I_1 =32, I2 =60 and I_3 =110 µmol photons $m^{-2}s^{-1}$, with three different light-dark cycles: 8:16h; 12:12h and 16:8 h and three salinities of 20, 30 and 40 psu. Experiments were performed in triplicate at 16 ± 2°C in air conditional room. Each replicate consists of one Petri dish .The growth of carposporelings was evaluated once a week, by measuring the increase of the diameter of the basal disc and the evolution of the percentage of erect fronds until four weeks,. The number of assessed carposporelings generally ranged from 10 to 15 per replicate.

****Juvenile tetrasporophyte cultivation***

After the carpospore germination and the erect frond emission, the sporelings were removed from Petri dishes to be cultivated, first in test tubes filled with the same culture medium until 116 days, under the same conditions. Only one carposporeling having erect frond was introduced in each test tube. This experiment phase was conducted in seven replicates. At the end, the carposporelings had been transferred in1000 ml glass flasks where they were cultivated until 228 days. The irradiance was fixed at 32 µmol photons $m^{-2}s^{-1}$. Culture medium was changed weekly. Growth in weight and in length was also observed once a week. Young plantlets (juvenile tetrasporophytes) were weighed with electronically Bosch scale and length was measured with a graduate ruler. The growth rate (GR) of the juvenile tetrasporophytes obtained was calculated following the growth formula,

$$GR = 100 \times \left(Log\left(W_T / W_{T_0} \right) \right) \times T^{-1}$$

where W_0 is the starting weight, W_t the ending weight and T (T_t-T_0) the number of cultivation days. The growth units are given as percent per day (% day^{-1}).The experiments in glass Erlenmeyers were performed in triplicate. Each replicate consists of one Erlenmeyer containing only one juvenile tetrasporophyte.

Agar extraction

At the end of the juvenile tetrasporophyte cultivation, wet alga was collected, weighed, oven dried overnight at 50 °C until constant weight. The agar extraction method followed was as follows: 50 mg of dried powered algae were placed in a test tube. 10 ml of H_2SO_4 (0.05 N) were added for 2 hours at room temperature. The powdered alga was then rinsed with distilled water.15 ml of NaOH (0.05 N) were added. The test tubes were kept in bath water at 90°C for 80mn, with continuous shaking. The filtration was performed under pressure and the filtrate was frozen at -20 °C for 24 hours. The filtrate was thawed using distilled water and kept at room temperature (20 °C) for 2 hours in order to obtain a thin film, which was collected and oven dried overnight at 105°C for 24 hours. The agar obtained was weighed and the agar yield was calculated as a percentage of seaweed dry weight. All experiments were performed in triplicate.

Data analysis

Data were analyzed using SPSS statistics 17.0 software. General Linear Model (univariate analyze of variance (ANOVA) and the Tukey multiple comparison test was performed to distinguish different results .Confidence level was set at 95 %.

[1] 40 psu was the natural seawater salinity during the study

RESULTS

Assessment of basal disc development and percentage of erect fronds

The diameter of basal disc in the sporelings of *G. gracilis* reached the highest growth when grown under 8:16 h light-dark cycles and the PFD of 60µmol photons.$m^{-2}s^{-1}$ and a salinity of 40 psu (262.86 ± 64.48 µm). At 12:12h and 16:8h light/dark, the results were lower whichever the irradiance level and the salinity were. The three combined tested factors (photoperiod, light intensity and salinity) had significant effects on the growth of basal discs. The interactions photoperiod *vs* light intensity; photoperiod *vs* salinity and light intensity *vs* salinity were also significant .

Two weeks after germination, the erect axes arose generally from the central basal disc in all the experiments (Fig.1 a,b,c). Erect fronds developed in a broad range of PFD, photoperiod and salinity. The highest percentage (100%) of erect fronds in the carposporelings of *G. gracilis* was recorded under the three tested irradiances and both short and long photoperiods (8:16h and 16:8h light/dark, respectively), combined with the salinity of 30 and 40 psu (Table I). Both the photoperiod and the PFD showed a significant effect on the percentage of erect fronds, while the salinity didn't (Table II). In addition, the interaction photoperiod *vs* PFD, photoperiod *vs* salinity, photoperiod *vs* PFD *vs* salinity was significant .The interaction PFD *vs* salinity was not significant (Table II).

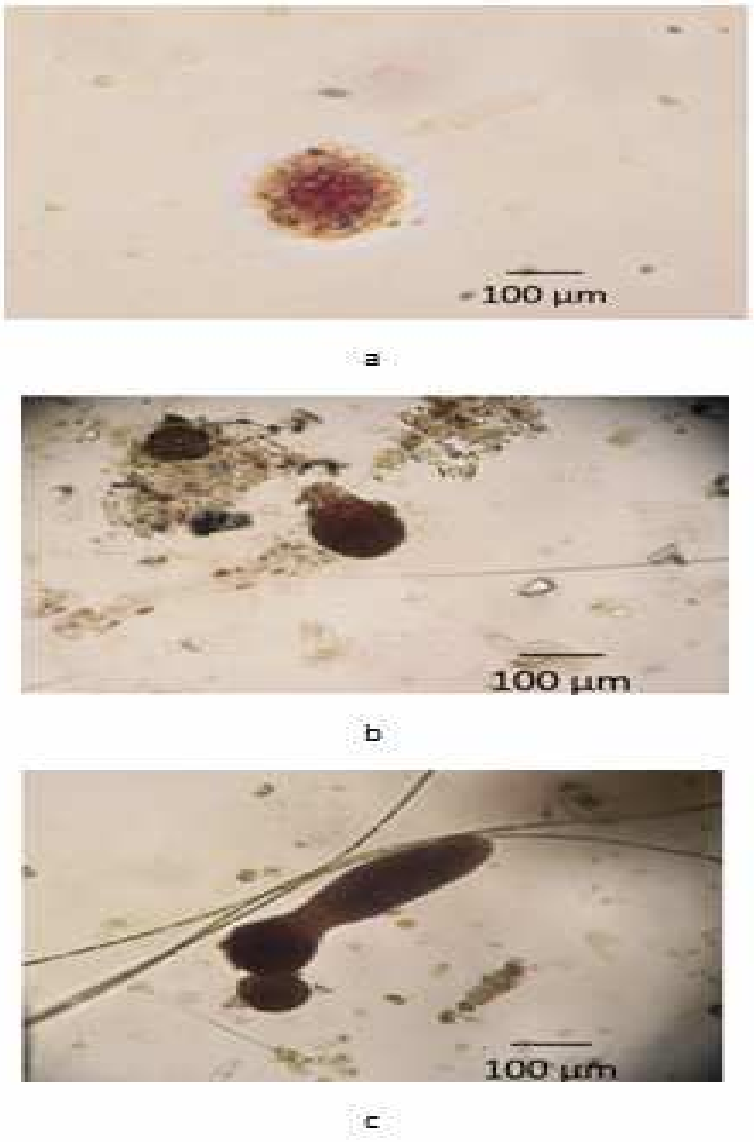

Fig.1: Different stages of carposporelings development in *Gracilaria gracilis* (a: after 10 days; b: after 15 days; c: after 28 days

Table I: Basal disc growth and percentage of erect fronds of carposorelings in *Gracilaria gracilis* under Photon Flux Density (PFD) of 32 , 60 and 110 µmol photons $m^{-2}s^{-1}$, photoperiod of 8/16; = 12/12; 16/8 and salinity of 20 psu; 30 psu; 40 psu for 4 weeks. Values are means ±SD (n, generally ranged from 30 to 40 carposporelings)

		Basal disc diameter (µm)			**Percentage of erect fronds (%)**		
Photon flux density (µmol photons $m^{-2}.s^{-1}$)		**32**	**60**	**110**	**32**	**60**	**110**
Photoperiod (h)	**Salinity(psu)**						
8 :16	20	162.76 ± 36.14	174.72 ± 46.28	149.76 ± 39.26	81.81 ±4.81	43.75 ±4.55	30.90 ±1.60
	30	166.66 ± 52.26	201.5 ± 63.44	132.86 ± 37.96	32.88 ±3.21	100.00 ±0.00	10.00 ± 1.20
	40	151.84 ± 50.28	262.86 ± 64.48	227.5 ± 48.62	73.53 ±8.39	66.66 ±5.54	100 ±0.00
12 :12	20	114.14 ± 21.84	0	0	25.80 ± 2.50	0	0
	30	151.06 ± 66.82	0	200.98 ± 55.9	44.0 ±3.50	0	91.43 ±8.80
	40	126.1 ± 27.82	124.8 ± 11.7	120.12 ± 23.92	14.28 ± 1.56	20.16 ± 3.72	62.96 ±6.58
16 :8	20	157.56 ± 59.28	110.5 ± 24.96	160.68 ± 51.48	70.9 ± 6.83	0	58.83 ± 4.56
	30	156.0 ± 42.38	126.62 ± 18.46	214.5± 61.62	100.00 ± 0.00	0	50.00 ±6.72
	40	162.24 ± 57.46	107.12 ± 6.5	138.32 ± 40.82	39.40 ± 3.60	0	21.05 ± 1.82

Table II: Three-way ANOVA of basal disc growth and percentage of erect fronds in the carposoprelings of *Gracilaria gracilis* cultivated under photoperiods of 8/16,12/12 and 16/8 , Photon Flux Densities (PFD) of 32,60 and 110 µmol photons $m^{-2}s^{-1}$ and salinity of 20,30and 40 psu for 4 weeks

Variable	**df**	**Basal disc**		**Erect frond**	
		F	**p-Value.**	**F**	**p-Value.**
Photoperiod	2	29.796	0,000	22.737	0.000
PFD	2	5.779	0.003	9.958	0.000
Salinity	2	5.562	0.004	1.086	0.345
Photoperiod *vs* PFD	4	11.773	0.000	8.506	0.000
Photoperiod *vs* salinity	4	3.401	0.009	4.842	0.002
PFD *vs* salinity	4	8.471	0.000	2.111	0.092
Photoperiod *vs* PFD *vs* salinity	8	6.122	0.000	3.048	0.007

Juvenile tetrasporophyte cultivation

Weight and length variation

The results showed that juvenile tertasporophytes grew well in a wide range of photoperiod and salinity. The photoperiod did not show a significant effect on the weight, while salinity did (TableIII). The interaction photoperiod *vs* salinity was not significant (Table III).At 8:16 photoperiod, the weight of

juvenile tetrasporophytes varied differently between the three tested salinities. The highest weight was obtained at 40psu (Fig.2A) involving a GR of 7.58±0.007% day^{-1}. At 12:12h light/ dark, the juvenile tetrasporophyte weight reached the maximum value at 40psu, involving a GR of 5.72±0.72% day^{-1} .At 16:8h light: dark cycles, the young plantlets' weight was comparable to that obtained at 8:16 h light: dark. The maximum of weight was also recorded at 40 psu and involving a GR of 6.94±0.007% day^{-1}.For the length of juvenile tetrasporophytes, the photoperiod did not have a significant effect, but salinity did (Table III). The interaction photoperiod *vs* salinity was not significant (Table III). At 8:16h light/ dark, the highest length was recorded at a salinity of 40psu (Fig 2 B).At 12:12h light/ dark, growth in length fluctuated generally between 12 and 14 cm. A relative high salinity (40psu) seems to be suitable for juvenile tetrasporophyte development and promote growth in length. At 16:8h light/ dark, the optimal growth in length was also recorded at 40 psu (Fig 2 B).

Agar yield

The results reported in Fig.3 showed that the optimal agar yield (30.65±1.2.1 % dw) was recorded both at the lowest photoperiod (8:16h light/dark) and salinity (20 psu). The algae cultivated at 12:12h and 16:8h light/ dark cycles reached closely similar results, both at 20 and 40 psu. Both photoperiod and salinity had a significant effect on the agar yield (Table III). The interaction photoperiod *vs* salinity was also significant (Table III).

Table III: Two-way ANOVA of juvenile tetrasporophyte weight, length and agar yield in *Gracilaria gracilis* cultivated under photoperiod of 8/16,12/12 and 16/8 , Photon Flux Density (PFD) of 32 µmol photons $m^{-2}s^{-1}$ and salinity of 20,30and 40 psu for 116 days in glass erlenmeyers

Variable	df	Weight		Length		Agar yield	
		F	p-Value	F	p-Value	F	p-Value
Photoperiod	2	2.010	0,190	0.184	0.835	22.787	0,000
Salinity	2	22.876	0.000	4.606	0.042	13.807	0.002
Photoperiod *vs* salinity	4	1.563	0.265	0.450	0.770	30.112	0.000

DISCUSSION AND CONCLUSION

The findings in this study revealed that the carposporeling development in *Gracilaria gracilis* was achieved differently at the three tested light and salinity conditions. The appearance of erect fronds occurred generally within two or three weeks and the phenomena continued later. In contrast, Komiyama & Sasamoto (1957) reported that sporelings of *Gracilaria verrucosa* grown in laboratory culture for two months did not develop any erect fronds. Bird et al. (1977) observed the erect fronds after two months at 15 °C, compared with others appeared within 10days at 25 °C or within 21 days at 20 °C. In addition, at 500 µW.cm-², 25°C and 12:12h or 10:14 h light: dark cycles, released carpospores attached and divided by a series of anticlinal and periclinal divisions. As a result, small multicellular discs have been formed within 10 days and then a small primary basal branching, which support our results.

Temperature and light were well known to be the critical factors determining spore germination and subsequent sporeling development. In the present study, the temperature was fixed at one level, while light (intensity and photoperiod) and salinity varied as described earlier. The results revealed in many cases a significant interaction between the three environmental factors on the development of basal discs of carposporelings and the appearance of erect fronds. Thus, a short photoperiod (8:16 h light/ dark) with an average irradiance level (60 µmol photons $m^{-2}s^{-1}$ and a relative high salinity (40 psu) led to have a large basal disc of the carposporelings. On the other hand, despite the absence of a significant effect of the salinity and no significant effect of the interaction PFD *vs* salinity, probably a long photoperiod (16:8 h light/dark cycles) and a low level irradiance of 32 µmol photons $m^{-2}s^{-1}$ and a salinity of 30 psu allowed to have the highest percentage of erect fronds. The same result was also recorded at 8:16 h light: dark cycles and both middle PFD and salinity. But in the latter case, the survival counted carposporelings was the highest. Our results showed that each stage of carposporeling development had a specific response to each environmental factor and also to their combined interactions. Indeed, the interactions among environmental variables are the rule rather than the exception (Lobban and Harisson, 1997). On the other hand, the chief biological parameters that condition a given plant's response to its environment are age,

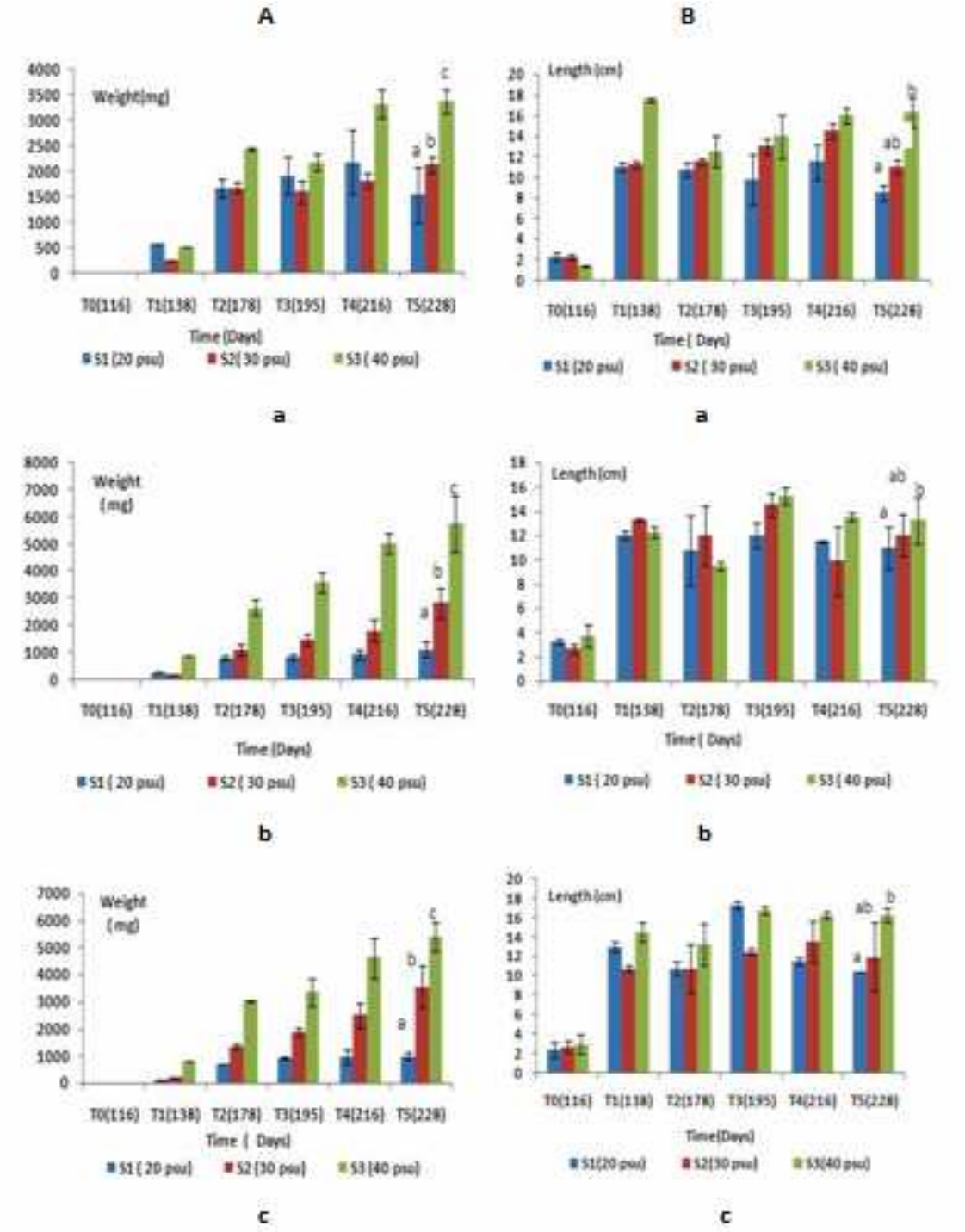

Fig.2: Growth in weight (A) and length (B) of juvenile tetrasporophytes in *Gracilaria gracilis* cultured under (a) a photoperiod of 8/16; (b) photoperiod of 12/12 and (c) of 16/8 photoperiod for 116 days

Values are means ±SD (n=2) of measured weights and lengths of juvenile tetrasporophytes of one plantlet per replicate. Different letters indicate significant differences according to the Tukey test

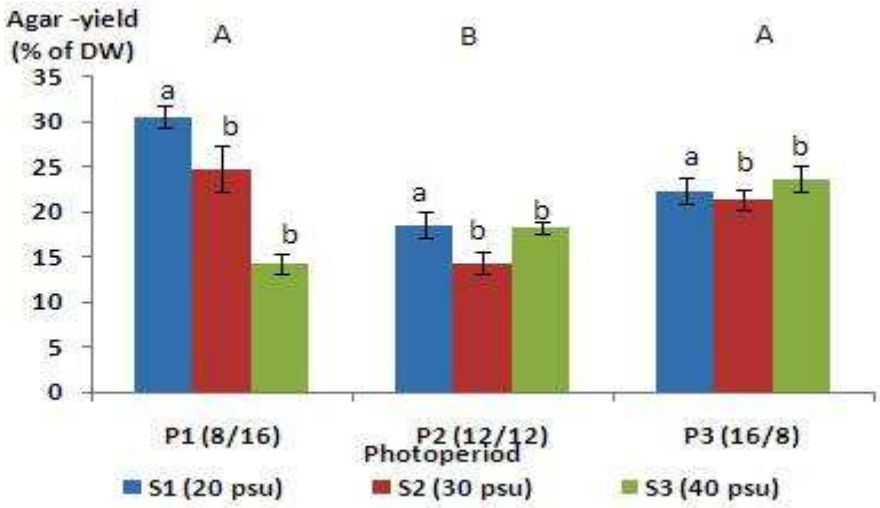

Fig. 3: Agar yield (% DW) of juvenile tetrasporophytes of *Gracilaria gracilis* cultured at photoperiods of 8/16, 12/12 and 16/8 and salinity of 20, 30 and 40 psu

Values are means ±SD (n=3) of measured agar yield of juvenile tetrasporophytes. Different letters indicate significant differences according to the Tukey test

reproductive condition, nutrient states, etc. (Lobban and Harisson, 1997). Our results agree with some of these remarks. In fact, the basal disc development in the carposporelings of *G. gracilis*, the appearance of erect fronds, their elongation and consequently the evolution of their percentage, tolerated a broad range of photoperiod, PFD, and salinity. Ramlov et *al.* (2012) found that carposporelings of *Gracilaria domingensis* (Kützing) Sonder ex Dickie 1874 (collected in Brazil) tolerated variations in salinity from 25 to 60 psu and basal discs and erect fronds developed better at a salinity of 30psu. This result is in agreement with our results. Nevertheless, the highest growth of basal disc of carposoprelings occurred under a higher light intensity (150 µmol photons $m^{-2}s^{-1}$). Despite the accurate assessment of erect frond length, this parameter ranged from 50 µm to 8.5 mm, after four weeks of culture. Yu *et al. (*2013) found that the carposporelings of *Gracilaria edulis* and *Gracilaria tenuistipitata* var *liui* both preferred lower salinity, while those of *Gracilaria tenuistipitata* var *liui* had a higher requirement for light. During the development of the juvenile tetrasporophytes, our results showed a significant effect of the salinity on the growth both in weight and in length. Neither the photoperiod nor the interaction photoperiod *vs* salinity had a clear significant effect on the growth of the juvenile tetrasporophytes. There was probably a significant influence of the salinity on the growth superior to the photoperiod because weight and consequently the GR achieved the highest value at the same salinity (40 psu), regardless of the photoperiod. Consentaneously with these results, a short photoperiod was sufficient to reach the best result. Padhi et al. (2011) obtained a GR ranging from less than 4 % to 8.96 % days $^{-1}$ in *Gracilaria verrucosa*. In the present study, the ash content of young tetrasporophytes was determined in one replicate because of the low amount of dry algae. Even though, the result showed that the highest contents were always obtained at a salinity of 40 psu, whichever the photoperiod and they ranged from 28.46 % to 41.09 %. The latter value was recorded under 8:16 h light:/dark. These results explained well the highest weight and consequently the highest GR that had been obtained. In other words, the latter was due to the accumulation of different salts in the tissues of carposporelings and after in the juvenile tetrasporophytes, when cultured in such conditions. Our findings are in agreement with previous studies (Israel et al. 1999), which suggested that the growth and chemical constituents were affected by concentration of salts and nutrients. The agar yield was affected by the photoperiod and the salinity. These two combined abiotic factors had a clear influence on the metabolism of agar synthesis in *G. gracilis*. In addition, while the salinity affected both the growth in weight and length and also the agar yield, the photoperiod had a contrasting effect on the two physiological responses. Moreover, a salinity of 40 psu led to have the highest GR, while a salinity of 20 psu led to reach the highest agar yield. This suggests that each expected response may be affected by specific levels of environmental factors. The latter may have either synergistically effects or inhibitory ones on the expected responses as mentioned and suggested by Lobban and Harisson (1997). Marine algae are notable for the large amount of several cell

compounds such storage polymers and cell wall-matrix polysaccharides (e.g. the agars). Such compounds are produced as a result of the photosynthesis in the seaweeds in response to several biotic and abiotic factors. Santelices and Doty (1989) reported that farming techniques for *Gracilaria*, either from spores or vegetative fragments, may provide the raw material for agar and agarose, by intensive labor means but at low cost in the less-developed countries (like Tunisia). The authors suggested that farming involving the out planting of spores indicates a need for applied *Gracilaria* spore biology.

In conclusion, the present study evidenced broad salinity tolerance of carposporelings and juvenile tetrasporophytes of *G. gracilis* and specific responses to light variation, namely the growth and agar yield. In this study, some interesting results were observed with respect to eco-physiology of *G. gracils*. Further studies are necessary to optimize the effects of more parameters on the growth and biochemical composition of the juvenile plantlets (tetrasporophytes) to have a solid platform of seaweed farming in Tunisia, both by spores and fragments.

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