Journal of Applied Biology & Biotechnology Vol. 11(1), pp. 55-60, Jan-Feb, 2023
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2023.110107

Effect of liquid seaweed (*Ulva rigida*) extract on the growth and rooting of carob (*Ceratonia siliqua* L.)

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ARTICLE INFO

Article history:
Received on: July 18, 2022
Accepted on: October 12, 2022
Available online: November 22, 2022

Key words:
Ulva rigida,
Ceratonia siliqua,
Roots,
In vitro growth,
Seaweed extract.

ABSTRACT

We investigated the *in vitro* effect of the use of *Ulva rigida* extract as a plant stimulant on carob root induction. Treatments at different concentrations of *U. rigida* extract were tested, as well as treatment by immersion of the basal portion of the hypocotylar sections in a solution of indole-3-butyric acid (IBA) before their cultivation in agar medium contains seaweed extract have been tested. The results showed that the application of *U. rigida* significantly increased the growth and rooting of carob cuttings. Plants treated with *U. rigida* extract outperformed the untreated plants in terms of plant height, number of internodes, number of leaves, number of roots, and root length with a high percentage of rooting of 83%. The higher response in the adventitious roots of carob hypocotyl cuttings was recorded with a treatment combination with IBA at a concentration of 2 mgL^{-1} and culturing in a medium containing 4% *U. rigida* extract. The results demonstrate a significant effect of *U. rigida* extracts on the root development of *Ceratonia siliqua*. This study highlights the possibility of developing procedures to improve the rooting capacity of recalcitrant plants while improving their growing environment.

1. INTRODUCTION

The carob tree (*Ceratonia siliqua* L.) is one of the most important ecological and socio-economic phylogenetic resources in Morocco. It is an endemic species of the Mediterranean basin that has numerous beneficial qualities and is the subject of numerous studies. Carob is highly valued in Mediterranean countries for its ornamental, nutritional and medicinal utility [1]. It is a long-lived evergreen and thermophilous tree that belongs to the family Fabaceae and forms an important part of Mediterranean vegetation [2]. The tree is well adapted to mild and dry areas with poor soils. Moreover, it is well known for its ability to adapt to adverse conditions such as drought, alkaline soils, heat, and dry atmosphere. These characteristics make this species widely used to prevent soil erosion and rehabilitation of degraded land in most Mediterranean countries [3].

Vegetative propagation is a promising method widely used for the mass production of selected and genetically uniform plants. However, the propagation of carob trees by cuttings is relatively uncommon. According to Hartmann and Kester [4], carob is among the most difficult species to root from cuttings, which restricts the mass production of selected elite genotypes using this method. While the use of exogenous auxins accelerates root initiation and increases the number and percentage of rooted cuttings, its proper application, availability, environmental impact, and costliness necessitate the development of an alternative way to stimulate carob cuttings to produce adventitious roots.

Since seaweed resources have been widely used in agriculture and horticulture, research on the benefits of seaweed products or macroalgae has been reported over the last 50 years [5]. Seaweed extracts could be a natural biostimulant treatment for improving plant growth and crop quality. Various seaweed extracts have been applied to several plants at different physiological growth stages and demonstrated a positive effect on seed germination, seedling growth, and enhanced physiological and biochemical activities on plants [6-7]. Seaweed extracts have been used to promote resistance to many diseases, increase plant resistance to drought, salinity, and heat stress, protect plants from aging by strengthening and supporting plant cells — especially fungal diseases and nematodes [8-10]. Several studies have shown that liquid extracts obtained from algae contain growth hormones (IAA and indole-3-butyric acid [IBA]), cytokinins, trace elements (Fe, Cu, Zn, Co, Mo, Mn, and Ni), vitamins, and amino acids which make these extracts applied in foliar sprays for several vegetative crops [11-13]. Recently, researchers proved that seaweed-based fertilizers are better and more economical than other fertilizers [14].

The present work aimed to evaluate the effect of *U. rigida* extract on the rooting capacity of carob hypocotyl cuttings. The effect of IBA was also studied.

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2. MATERIALS AND METHODS

2.1. Seaweed Collection

Seaweed (*Ulva rigida* C. Agardh) was collected froman intertidal rocky shore zone in Azla (Tétouan, Mediterranean Sea, Northern Morocco: 35° 32.2 N, 05° 14.7 W) at a depth of 0.5–1 m during July and August. First, the algae were washed with sea water to remove macroscopic epiphytes and sand particles and subsequently the algae were again washed with tap water to remove the adherent salt. For 4 days, the samples were air dried at 25°C, followed by thermostat drying at 60°C for 12 h. The seaweed was washed with seawater and brushed extensively.

2.2. Preparation of Liquid Seaweed Extract (LSE)

First, the dried seaweed was crushed manually and powdered with an electric grinder. The seaweed powder was then diluted with sterile distilled water at a ratio of 10: 100 (w/v) and then heated in a water bath at 60°C for 20 min. Second, the extracts were filtered on filter paper and sterile Millipore (0.45 µm). The resulting extract was taken as a 100% concentration of the LSE and stored at 4°C for further experimental studies. Different concentrations of LSE were prepared (1, 2, and 4%) [15].

2.3. Experimental Design and Treatments

The carob seeds were sacrificed by sulfuric acid (95%) for 15 min and subsequently the seeds were disinfected with 0.01% of mercury chloride ($HgCl_2$) for 30 min. Seeds should be rinsed in sterile distilled water (SDW) 3 times before being germinated on jars containing SDW solidified with 0.7% agar and autoclaved at 1 bar and 120°C for 20 min. The cultures were incubated in the light in a growth chamber at 25 ± 2°C and 2000 lux for 16 h, with light provided by white fluorescent lamps (Philips TL MRS 40 W/54-765)/8 h. After 2 weeks of *in vitro* germination, hypocotyl cuttings were taken from 10-day-old seedlings by cutting the stem above the root collar.

Different concentrations of LSE (1, 2, and 4%) were added to agar water and used as the base medium. The explants were subcultured onto the prepared media and maintained in a culture chamber at 25 ± 2°C and 2000 lux for 16 h, with light provided by white fluorescent lamps (Philips TL MRS 40 W/54-765)/8 h.

2.4. Effect of IBA Treatment and Seaweed Extract on Rooting

To improve the root response, the basal ends of the hypocotylidal sections were immersed in a solution containing IBA at three different concentrations (0.5, 1, and 2 mgL^{-1}) for 12 h in darkness at 5°C, the cuttings were subsequently rooted in an agar mediums supplemented with extract of *U. rigida*. Agar medium without seaweed extract was used as a control.

Hypocotyl cuttings were grown for 4 weeks in a growth chamber under the conditions described for seed germination.

2.5. Statistical Analysis

Each treatment was repeated 3 times, with 30 cuttings per replicate. After 4 weeks of culture, the following carob tree growth parameters were measured: Plant height, stem length, number of internodes, number of leaves, number of buds, percentage of rooting, number of roots per plant, and roots length. The data were analyzed using ANOVA tests and the means were compared using Duncan's *post hoc* test at the 5% probability level.

3. RESULTS

3.1. Effect of *U. rigida* Extract on the Growth of Carob Cutting

The results presented in Table 1 indicate that applying seaweed extract has a positive effect by stimulating the growth parameters of carob hypocotyl cuttings. Thus, the cuttings cultured with *U. rigida* extract had a vigorous and qualitative appearance when compared to controls.

The data presented in Figure 1 demonstrate that all examined concentrations of *U. rigida* extract had a significant effect on plant height, stem length, number of leaves, and number of internodes. Moreover, the stimulation of bud formation was remarkably higher compared to untreated control. The cuttings continued to grow on culture medium for over 4 weeks in the presence of *U. rigida* extracts. The most important stimulatory effect of *U. rigida* extract was observed using a concentration of 4% when compared to the untreated control.

3.2. Effect of *U. rigida* Extracts on the Rooting of Carob Cuttings

Root formation in carob cuttings was also enhanced by *U. rigida* extracts under *in vitro* conditions. Based on the results presented in Table 2, ANOVA revealed significant differences between experimental treatments with seaweed extract in the culture medium for the percentage of rooting, number of roots, and root length. Figure 2 demonstrates the favorable effect of using seaweed extract at a 4% concentration on rooting (83.33%) when compared to the control (30%).

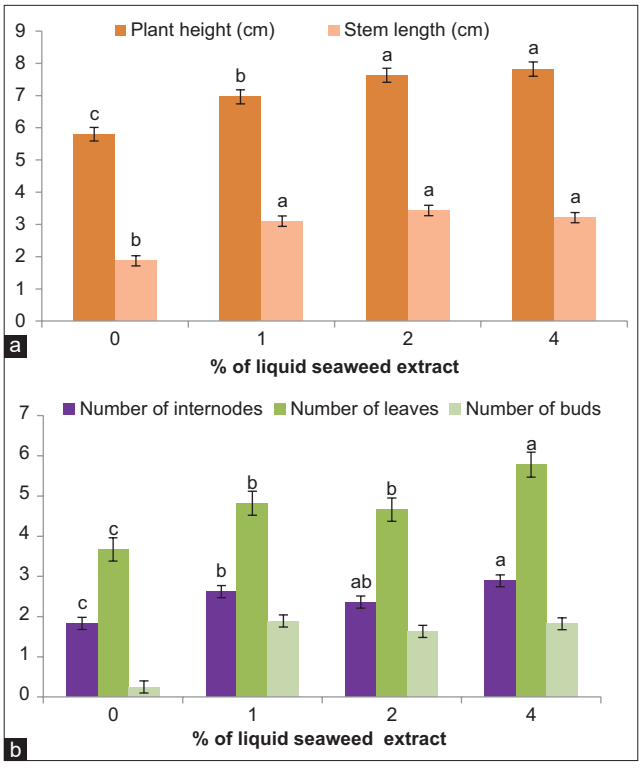

Figure 1: Effect of *Ulva rigida* extracts on the growth of carob plants. Mean values of plant height, stem length, number of internodes, number of leaves, and number of buds after 4 weeks of culture. Means followed by the same letter within a treatment group werenot significantly different at $P < 0.05$ using Duncan's multiple range tests. LSE: Liquid seaweed extract.

Table 1: Effects of *Ulva rigida* extracts on the growth of *C. siliqua*. Mean values of plant height, stem length, number of internodes, number of leaves, and number of buds after 4 weeks of culture.

LSE concentration (%)	Plant height (cm)	Stem length (cm)	Number of internodes	Number of leaves	Number of buds
Control (0)	5.80±0.21^{c}	1.87±0.16^{b}	1.83±0.15^{c}	3.67±0.29^{c}	0.25±0.15^{b}
1	6.96±0.22^{b}	3.10±0.16^{a}	2.62±0.15^{b}	4.82±0.30^{b}	1.89±0.15^{a}
2	7.63±0.22^{a}	3.43±0.16^{a}	2.36±0.15^{ab}	4.66±0.29^{b}	1.633±0.15^{a}
4	7.82±0.22^{a}	3.21±0.16^{a}	2.89±0.15^{a}	5.78±0.31^{a}	1.821±0.15^{a}

Means followed by the same letter within a treatment group were not significantly different at $P<0.05$ based on Duncan's multiple range tests. LSE: Liquid seaweed extract

Table 2: Effects of *U. rigida* extracts on the rooting of carob plants. Mean values of rooting rate, number of roots, and root length after 4 weeks of culture.

Concentration % of LSE	% Rooting	Number of roots	Root length (cm)
Control (0)	30^{c}	2.03±0.56^{b}	0.38±0.23^{b}
1	70^{b}	3.93±0.58^{a}	1.06±0.24^{a}
2	76.66^{a}	4.30±0.57^{a}	1.26±0.23^{a}
4	83.33^{a}	5.46±0.59^{a}	1.75±0.24^{a}

Means followed by the same letter within a treatment group were not significantly different at $P<0.05$ based on Duncan's multiple range tests. LSE: Liquid seaweed extract

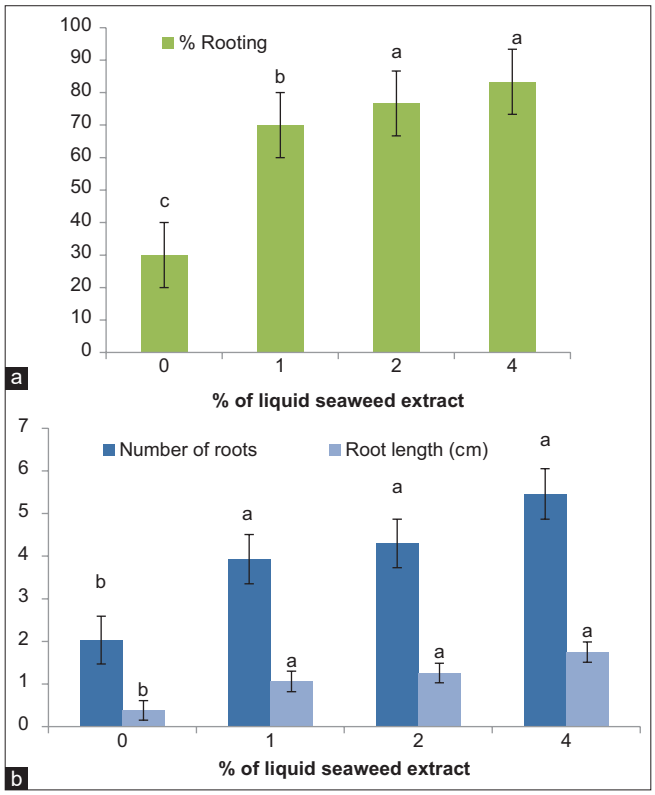

Figure 2: Effect of *Ulva rigida* extracts on the rooting of carob plants. Mean values of rooting rate, number of roots, and root length after 4 weeks of culture. Means followed by the same letter within a treatment group were not significantly different at $P < 0.05$ based on Duncan's multiple range tests. LSE: Liquid seaweed extract.

3.3. Effect of IBA Treatment and Seaweed Extract on Rooting

The effect of MG medium supplemented with different concentrations of *U. rigida* extracts combined with pretreatment using IBA at different concentrations of (0.5, 1, and 2 mgL^{-1}) on plant rooting was examined [Table 3]. After 4 weeks of culture, the optimal results in terms of plant length, number of roots, and length of root were provided with media containing a high concentration of *U. rigida* extract (4%) and a high concentration of IBA (2 mgL^{-1}). The data presented in Figure 3 demonstrates that the rooting capacity of carob cuttings was remarkably higher with the combination treatments involving exogenous IBA and *U. rigida* extract in culture medium when compared to the non-treated ones [Figure 4].

4. DISCUSSION

The application of *U. rigida* extract significantly enhanced the growth, and rooting capacity of carob hypocotyl cuttings. These results are in agreement with those of previous studies using seaweed extract to stimulate the growth of crops such as mung bean [16], cucumber [17], Sour Orange [18], and lettuce [19]. A chemical analysis of algae and their extracts noted the presence of carbohydrates, macro and microelements, phenylacetic acid, vitamins, and plant growth regulators such as auxin and cytokinins [20-23]. This leads to growth stimulation and acceleration in the roots, and — when combined with cytokinins — produces a larger root mass [24]. A larger root mass provides better water and mineral uptake as well as healthier, more stress-resistant plants [25]. Seaweed extracts are rich in cytokinins that stimulate rapid cell division and the production of new cell walls.

Furthermore, the application of IBA improved the rooting percentage and root number when compared to untreated control. IBA auxin was appropriate for the effective rooting of cuttings at different concentrations. However, the previous reports on carob micropropagation demonstrated that using an IBA-enriched MS medium can provide rapid root initiation [26,27]. The efficacy of IBA in different plant species compared to other auxins in inducing rooting of shoots has also been reported [28,29]. Irrespective of the IBA treatment, the presence of *U. rigida* extracts in media served a crucial role in the promotion of rooting in all treatments. The results indicate that growth media type played a vital role in the rooting of cuttings. The results of the present study indicate that the use of *U. rigida* extracts in a culture medium combined with IBA pretreatments caused a significant increase in number roots and root length than when compared to the treatment of hypocotyl bases with IBA alone. The beneficial effect of natural molecules on Soil-Plant Systems was shown by El Boukhari *et al*. [30] and Kurepin *et al.* [31]. The latter author reported that the release of secondary metabolites and their assimilation was much more important as naturally biostimulators.

Additional studies of various algae have shown that the stimulating effect of root growth was more pronounced when extracts were applied at an early stage of corn growth and exhibited a response similar to that of auxin. Using algae extracts as a biostimulant can affect root development by improving lateral root formation and increasing total root system volume [32-35].

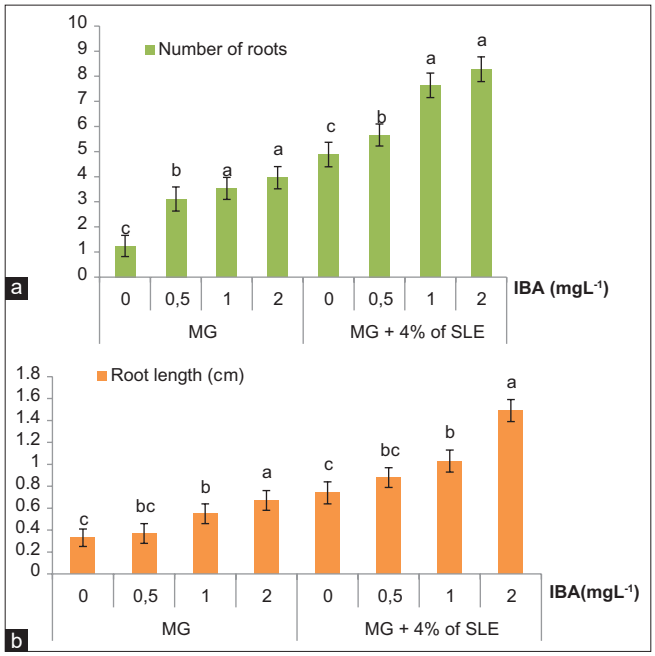

Figure 3: Effect of different concentrations of IBA hormone combined with *Ulva rigida* liquid seaweedextract in medium culture on the number of roots and root length of carob plants after 4 weeks of culture. Means followed by the same letter within a treatment group werenot significantlydifferent at $P < 0.05$ based on Duncan's multiple range tests. MG: Distilled water solidified with 7.0 gL^{-1} agar– LSE: Liquid seaweed extract.

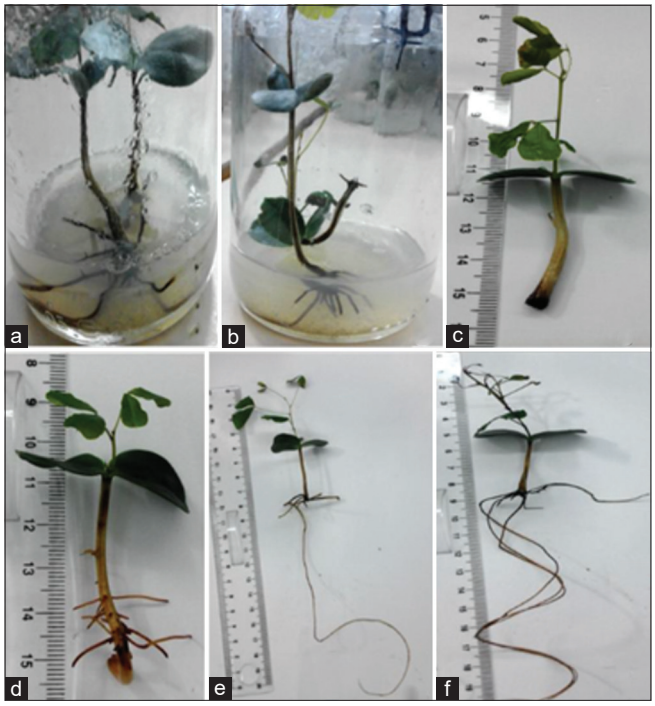

Figure 4: (a-b-d) Abundant rooting after 4 weeks of cultivation in agar medium contains different concentrations of liquid seaweed extract (1; 2; 4%). (c) Shoots remain uprooted after 4 weeks in agar media. (e and f) Abundant rooting with longer roots after 4 weeks of culture in agar media contain 4% of seaweed liquid extract associated by treatment with 2 mgL^{-1} of IBA.

Table 3: Effects of different concentrations of IBA treatment combined with the use of *Ulva rigida* liquid seaweed extract in medium cultures on plant height, number of roots, and root length of carob plants after 4 weeks of culture.

Medium	IBA concentration (mgL^{-1})	Plant height (cm)	Number of roots	Root length (cm)
MG	0	5.33±0.19^{b}	1.24±0.42^{c}	0.33±0.08^{c}
	0.5	5.78±0.22^{a}	3.11±0.48^{b}	0.37±0.09^{bc}
	1	5.59±0.20^{a}	3.53±0.44^{a}	0.55±0.09^{b}
	2	5.63±0.20^{a}	3.96±0.44^{a}	0.67±0.09^{a}
MG + 4% of LSE	0	6.70±0.22^{b}	4.88±0.49^{c}	0.74±0.10^{c}
	0.5	7.15±0.20^{a}	5.66±0.44^{b}	0.88±0.09^{bc}
	1	7.86±0.22^{a}	7.64±0.49^{a}	1.03±0.10^{b}
	2	8.02±0.22^{a}	8.28±0.49^{a}	1.49±0.10^{a}

Means followed by the same letter within a treatment group werenot significantly different at $P < 0.05$ based on Duncan's multiple range tests. MG: Distilled water solidified with 7.0 gL^{-1} agar, LSE: Liquid seaweed extract

In the present *in vitro* study, no contamination was recorded. It is also thought that seaweed extract helps protect cuttings against bacterial or fungal infections and keeps plants healthy and strong.

In addition to its rooting difficulties, the carob tree is a recalcitrant plant. Thus, scientific research is currently being conducted to increase carob tree horticulture and improve conditions for its rooting. Notably, several studies have been conducted in this context to improve the rooting capacity of carob (*Ceratonia siliqua* L.) cuttings using mycorrhizal fungi and ultrasonic [36,37].

Applying extract derived from algae as a natural and ecological biostimulant is a promising technique that can have positive effects on plant development.

5. CONCLUSION

The present study confirms that *U. rigida* extract can serve as a natural fertilizer and biostimulant to improve the growth and rooting of carob and many other recalcitrant plants. The beneficial effect of seaweed extract application presented in this study highlight sits potential for the development of a successful and sustainable method to improve plant rooting that could offer viable field applications.

6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

7. FUNDING

This work was supported by internal funding from Abd Elmalek Essaadi University, Morocco.

8. CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

9. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

10. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

11. PUBLISHER'S NOTE

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How to cite this article:
Zouari N, Bougdaoua H, El Mtili N. Effect of liquid seaweed (*Ulva rigida*) extract on the growth and rooting of carob (*Ceratonia siliqua* L.). J App Biol Biotech. 2023;11(1):55-60. DOI: 10.7324/JABB.2023.110107