Aquaculture International (2026) 34:42
https://doi.org/10.1007/s10499-026-02442-0

RESEARCH

Biostimulant effect of seaweed extracts on micropropagation of important ground macrophyte *Micranthemum tweediei* and data-driven insights using machine learning and response surface regression

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Received: 17 October 2025 / Accepted: 6 January 2026 / Published online: 23 January 2026
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Abstract

Commercially viable in vitro cultivation of ornamental and aquatic plants depends heavily on the proper use of bio-stimulants that encourage healthy and uniform growth. This study explores the role of two seaweed extracts, *Jania rubens* and *Cystoseira barbata*, in promoting in vitro micropropagation of *Micranthemum tweediei* (Monte Carlo), a widely used aquatic plant. Culture medium was enriched with different concentrations (2.5–20.0%) of *J. rubens* and *C. barbata* extracts with 1–4 g L^{-1} Murashige and Skoog (MS) nutrient media. Considering all results, *J. rubens* extract was more effective than *C. barbata* in terms of micropropagation and chlorophyll content of *M. tweediei*. Addition of 5% *J. rubens* extract to 2 g L^{-1} MS medium (approx. half strength) resulted in a 47% increase in clump diameter (5.18 cm), a 173% increase in rooting rate (92.4%), and a 108% increase in shoot clump fresh weight (3.6 g), and 84%, 115%, and 87% increases in chlorophyll *a*, *b*, and total chlorophyll levels, respectively, compared to the control 2 g L^{-1} MS medium without extract. Micropropagation parameters resulting from 4 g L^{-1} MS (approx. full strength) supplemented with *J. rubens* extract were lower than those obtained from 2 g L^{-1} MS. Unlike *J. rubens*, *C. barbata* showed the highest micropropagation when 10% extract was added to 4 g L^{-1} MS nutrient medium, resulting in a clump diameter of 5.11 cm, 42.8% rooting, a fresh clump weight of 7.37 g, and a dry clump weight of 0.59 g. Propagated in vitro plants were easily acclimated to external conditions in aquariums. Results of response surface regression analysis (RSRA) confirmed the results, and MS concentration influenced the plant biomass and chlorophyll contents, whereas seaweed type and concentration regulated the clump diameter and rooting. Application of machine learning models validated and predicted the outcomes precisely. Multi-layer perceptron (MLP) model exhibited superior predictive accuracy for morphological traits, while more predictive accuracy of chlorophyll parameters was attributed to Random Forest (RF) model. This study has shown for the first time that aquatic plants can be micropropagated more successfully and at lower cost by adding seaweed extracts to in vitro nutrient media without the need for growth regulators.

Handling Editor: Ronan Sulpice

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Keywords Macroalgae · *Jania rubens* · *Cystoseira barbata* · Micropropagation · Chlorophyll *a/b*

Introduction

Seaweeds, known as macroalgae, have thousands of species and are broadly classified into three main groups according to their pigmentation (Abdel Latef et al. 2017; Baweja et al. 2019): Phaeophyta (brown), Rhodophyta (red), and Chlorophyta (green). Due to their rich phytocompounds and nutrient content, they have found an important use in the fields of food, feed, agriculture, pharmacy, medicine, and industry (Rifi et al. 2022; Arioli et al. 2024; Mughunth et al. 2024). Today, extracts obtained from many seaweed species are commercialized and used as fertilizer in agriculture. The application of these extracts in plant production increased crop yield, root and leaf development, flowering and fruit set, and tolerance to biotic and abiotic stress conditions (Pereira and Cotas 2019; Khedia et al. 2020; Mukherjee and Patel 2020; Nanda et al. 2022; Mondal et al. 2025; Prakhyath et al. 2025).

Jania rubens (L.) Lamx, belonging to the Corallinaceae family of red algae (Rhodophyta), is widely distributed in the North East Atlantic, Indian Ocean, China Sea, Mediterranean, and Black Sea (Boran et al. 2024). *Cystoseira* is a genus of dominant brown algae that grow widely throughout the Atlantic, Mediterranean, and Black Sea regions (Bruno de Sousa et al. 2019). Red and brown seaweeds, including *J. rubens* and *Cystoseira barbata* (Stackhouse) C. Agardh, are rich in macro (Na, K, P, Mg, and Ca) and micro-nutrients (B, Zn, Fe, and Mn), complex minerals, and plant growth regulators (PGRs) such as 6-benzylamino purine, kinetin, zeatin, indole-3-acetic acid, gibberellins, ethylene, jasmonates, salicylic acid, strigolactones, and brassinosteroids (Stirk et al. 2014; Satish et al. 2015; Yalçın et al. 2019; Anbazhakan et al. 2022; Mughunth et al. 2024). El-Din and El-Ahwany (2016) reported that *J. rubens*, in particular, has high carbohydrate (14.48%), protein (11.3%), Na (20.9 mg/g), K (3.3 mg/g), Ca (423.4 mg/g), Mg (29.9 mg/g), and Fe (0.48 mg/g) content. In addition, they also contain significant amounts of protein, fat, polysaccharides, fiber, amino acids, antioxidants, phenols, flavonoids, carotenoids, betaines, and vitamins K, E, and B (El-Chaghaby et al. 2019; Trica et al. 2019; Rashad and El-Chaghaby 2020; Rifi et al. 2022; Mughunth et al. 2024). These rich contents make *J. rubens* and *C. barbata* very important in terms of plant nutrition and production. Studies have also reported that they have antitumor and antibacterial effects as well as wound healing, antidiabetic, anti-inflammatory, and antioxidant properties (Gheda et al. 2018; Sellimi et al. 2018; Krupnik et al. 2023; Nabil-Adam et al. 2023; Boran et al. 2024; Rifi et al. 2022).

With tissue culture techniques, it is possible to produce millions of commercial plants with the same genetic structure and free from diseases in a short time (Özcan et al. 2023, 2025). However, the most important problem with in vitro techniques is the high cost of nutrients and plant growth regulators (PGR) used in the nutrient media. Extracts from different seaweeds such as *Fucus spiralis*, *Cystoseira myriophylloides*, and *Laminaria digitata*, and microalgae *Desmodesmus subspicatus* have been added to the nutrient media to enhance in vitro plant development in *Solanum melongena* (Satish et al. 2015), *Nicotiana benthamiana*, grape, plum, and apricot (Esserti et al. 2017), *Plumbago zeylanica* (Kakade et al. 2022), and *Cattleya warneri* (Navarro et al. 2023). Additionally, fucoidan fractions extracted from *Turbinaria decurrens* were used to enhance in vitro seed germination, organogenesis, and adventitious root development in finger miller and eggplant plants (Kaniyassery et al. 2024). In our very recent study, we also observed that *J. rubens* and *C. barbata* extracts increased the in vitro

micropropagation of the important water/aquarium *Anubias barteri* and *Alternanthera reineckii* (Özcan and Atar 2025).

Micranthemum tweediei Benth., also known as Monte Carlo, grows naturally in wet areas and along stream banks in Argentina, Uruguay, and Southern Brazil. Its leaves, which have a round or elliptical leaf structure, are smaller and darker green than *M. umbrosum*. *M. tweediei* has been one of the most used ground-covering plants in aquariums in recent years. It forms a compact mat due to its ability to grow by crawling in the immersed and submerged form, which gives aquariums a very beautiful appearance in terms of landscape aesthetic. While there is no data available on the global trade of *M. tweediei*, it is very actively traded in European, North American, African, Middle Eastern, and Asian markets. Therefore, *M. tweediei* is readily available in local pet shops, online e-commerce sites, hobby forums, and among international plant sellers in small in vitro containers with a price of $6–12. As this plant is planted in large quantities on the aquarium ground, it is crucial to obtain it in larger culture dishes and at a lower price. Aquarium hobbyists, especially in developing countries, often find it unaffordable due to its high price. The best approach in the production of low-cost in vitro plants is to reduce or eliminate the use of nutrient media and plant PGRs, which account for approx. 25–30% of the cost. Therefore, the present study was aimed to in vitro micropropagate the most used ground-covering macrophyte *M. tweediei* efficiently by adding *J. rubens* and *C. barbata* extracts to nutrient media and to reduce or eliminate the use of media and PGRs.

Advancement in plant biotechnology demands the need of adopting modern computational tools for data analysis and optimization. Use of response surface methodology (RSM) and machine learning (ML) models is increasing substantially for optimization and predictive analysis, especially in plant tissue culture in recent years (Ali and Aasim 2024). RSM is a statistical tool which helps to design the multiple input variables with a lesser number of experimental runs to optimize the variables by employing a quadratic polynomial equation to the data (Lamidi et al. 2022; Younis et al. 2022). However, highly nonlinear and complex data sets often result in false or reduced prediction efficiency. The problem can be solved with state-of-the-art computational tools like machine learning algorithms that are used for predictive modeling and pattern recognition in the given data set (Ali and Aasim 2024; Kyriazos and Poga 2024). ML models do not rely on predefined functional relationships between input variables and output responses, which makes them suitable for identifying or capturing nonlinear dependencies and interactions in datasets (Kyriazos and Poga 2024). In recent years, the use of both RSM and ML models in plant tissue culture has been prevailing due to offering a more robust approach for understanding the complex biological systems exhibiting nonlinear behavior due to multiple factors (Özcan et al. 2023). In this study, the hybrid RSM-ML approach was used for optimizing and predicting the impact of two different types of extracts of algae, applied at four different concentrations with four different concentrations of Murashige and Skoog (MS) medium. Three different ML models were used for data validation and prediction with the help of six different performance metrics.

Materials and methods

Seaweed collection and extract preparation

The red alga *Jania rubens* was collected from the Aegean Sea coast of Urla, Izmir, and the brown alga *Cystoseira barbata* was collected from the Black Sea coast of Trabzon, Türkiye, from June to September. Identification of these species was made by cross-referencing

with taxonomy books and reference herbaria. The collected *J. rubens* and *C. barbata* samples were first washed with sea water and then placed in containers containing sea water and brought to the laboratory in a cold chain. After being washed 5 times with tap water in the laboratory, they were finally rinsed with distilled water. The washed seaweed samples were dried in the open air in the shade for 7 days by mixing occasionally and then roughly ground with an electric mill. The extraction process was carried out with minor modifications as described previously (Satish et al. 2015; Kakade et al. 2022). Three liters of sterile distilled water was added to 500 g of seaweed powder and autoclaved at 121 °C for 30 min under 1.4 kg/cm^2 pressure. The autoclaved samples were passed through a muslin cloth and left to cool at room temperature. Then, after being filtered with Whatman filter paper no. 1, the liquid extract was stored at 4 °C and used as a stock solution.

Media preparation and culture conditions

The recommended amount of MS (Duchefe Biochemie, product code: M0222) nutrient medium (Murashige and Skoog 1962) fo*r* in vitro culture is 4.4 g L^{-1}. In the present study, MS-free and 1, 2, and 4 g L^{-1} MS media with the addition of 2.5, 5.0, 10, and 20% *J. rubens* and *C. barbata* extracts were tested for micropropagation of *M. tweediei*. MS-free nutrient medium did not contain any MS minerals and vitamins. MS media with varying concentrations and without extract were used as controls for the tested extract ratios. Considering the rich nutritional content of seaweeds and the results of previous studies with aquatic plants (Özcan et al. 2023, 2025), 20 g L^{-1} sucrose and 1.5 g L^{-1} agar were added to these media after adjusting the pH of the media to 5.8 with 1 N HCl or NaOH; they were autoclaved at 121 °C under 1.4 kg/cm^2 pressure for 20 min.

Explant culture and micropropagation

Micranthemum tweediei plantlets purchased from local vendors' aquariums were first surface sterilized for 15 min in 20% commercial bleach containing 4.5% w/v sodium hypochlorite with 1–2 drops of Tween 20 (Caisson Labs). Then, the plantlets were rinsed with sterile water 3 times and cultured on nutrient medium for in vitro propagation and stock formation in glass jars. Small clumps containing approximately 10 shoots were taken from these stock cultures with the help of forceps and placed on the prepared nutrient media. Four explants were cultured in 100 × 20 mm glass petri dishes, resulting in each treatment containing three petri dishes and a total of 12 explants. All cultures were incubated in growth cabinets under cool white fluorescent light (35 µmol photons m^{-2} s^{-1}) in a 16-h photoperiod at 24 ± 1 °C. Clump diameter, rooting rate, and fresh and dry weight of *M. tweediei* clumps were determined in the cultures developed 4 weeks after the beginning of culture. The rooting rate is calculated as the ratio of rooted shoots within a clump to the total number of shoots.

Estimation of chlorophyll content

Chlorophylls *a*, *b* and total chlorophyll content of *M. tweediei* in vitro shoots were estimated by the spectrophotometric method with Shimadzu UVmini-1240 with minor modifications (Hipkins and Baker 1986; Shetty et al. 1996). Fifty milligrams of green fresh material was placed in 3 mL of 100% methanol (Merck) in 15 mL sterile Falcon tubes with caps and incubated for 2 h at 23 °C in the dark with three replications. After the samples were

mixed, 1.5 mL of the methanol fraction was taken, and the absorbance values were measured in the spectrophotometer at 650 and 665 nm. The chlorophyll contents of the samples were calculated as µg chlorophyll/g fresh tissue using the following formulas:

$$\begin{aligned} Chlorophyll\ a &= (16.5 \times A665 - 8.3 \times A650) \times 3/0.05 \\ Chlorophyll\ b &= (33.8 \times A650 - 12.5 \times A665) \times 3/0.05 \\ Total\ chlorophyll &= (25.8 \times A650 + 4.0 \times A665) \times 3/0.05 \end{aligned}$$

A650 and A665 are the absorbance values obtained at 650 and 665 nm.

Statistical analysis

All experiments consisted of three petri dishes (replicates), each containing four explants. The experiments were repeated at least once in this manner, and all results were pooled. The data obtained were subjected to analysis of variance (ANOVA), and the significance between the means was determined with the Tukey test. Results were further computed with response surface regression analysis (RSRA) to investigate the impact and relationship between input and output variables. Results were also analyzed with Pareto charts and normal plots to validate the statistical assumptions and correlation. RSRA, Pareto chart, and normal plot analysis were computed with Minitab 21.0 statistical program.

Machine learning modeling

In this study, data generated after the experiment and analyzed with RSRA was further validated and predicted by machine learning models. Decision-tree based Random Forest (RF) (Pavlov 2000), Extreme Gradient Boosting (XGBoost) (Chen and Guestrin 2016), and neural network-based multilayer perceptron (MLP) models were employed for data analysis. The RF model is based on bagging or bootstrap aggregation and trains many trees simultaneously and predicts outcomes (Eq. 1).

$$y = \sum_{\mathrm{i}=1}^{\mathrm{n}} (\alpha_{\mathrm{i}} - \alpha_{\mathrm{i}}^{*}) k\left(x, x_{\mathrm{i}}\right) + b \tag{1}$$

y = observed value of the data point, n = number of samples.

XGBoost is a boosting-based model that creates a group of sequential tree models and derives results from the overall results, and it needs the reduction of the objective function (Chen and Guestrin 2016). The objective function of XGBoost that needs to be lowered is shown in Eqs. 2 and 3, and it also illustrates the model at the jth iteration.

$$y_{\mathrm{i}} = F\left(x_{\mathrm{i}}\right) = \sum_{(\mathrm{d}=1)}^{\mathrm{D}} f_{\mathrm{d}}\left(x_{\mathrm{i}}\right), f_{\mathrm{d}} \in F, i = 1, \ldots, n \tag{2}$$

$$L_{\mathrm{j}} = \sum_{(\mathrm{i}=1)}^{\mathrm{n}} l(y_{\mathrm{i}}, \hat{y}_{\mathrm{i}}^{(\mathrm{j}-1)} + f_{\mathrm{j}}\left(x_{\mathrm{i}}\right)) + \Omega\left(f_{\mathrm{j}}\right) \tag{3}$$

MLP is a feedforward neural network comprised of multiple layers of processing nodes that are connected in a feedforward manner. The model is completely interconnected with one or more layers of input, output, and hidden layers. The backpropagation method is used

to train the data to update the error-related weights and biases (Katırcı et al. 2021) until lowering Eq. 4.

$$E = \frac{1}{K}\sum_{k=1}^{K}(y_k - \hat{y}_k)^2 \tag{4}$$

y: observed value of data point k, k: number of samples.

Leave-one-out cross-validation (LOO-CV) method (Webb et al. 2010) was used for investigating the performance of models (Webb et al. 2010), where the number of instances as folds is the same in the dataset. This technique is more novel and efficient for small sample sizes, and training and testing are performed with the remaining instances and the selected instance. After cross-validation, the grid search technique was employed for optimizing the hyperparameters. Coding of the whole analysis was done with the aid of the Sklearn package (Pedregosa et al. 2011) and the open-source Python programming language (Van Rossum and Drake 2009). Different performance metrics were used for investigating the model's performance. Performance metrics are highly critical for evaluating the reliability and predicting the accuracy of tested models. In this study, six different performance metrics were used for validation and prediction of complex GE interactions.

The coefficient of determination (R^2) plays a crucial role in regression-based machine learning analyses, as it quantifies how much of the variance in the dependent variable is explained by the model. Values approaching 1.0 suggest high predictive reliability and are expressed mathematically as follows:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(Y_i - \widehat{Y}_i)^2}{\sum_{i=1}^{n}(Y_i - \widetilde{Y})^2} \tag{5}$$

Root mean square error (RMSE) presents the prediction errors, and low scores demonstrate the better model accuracy.

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(Y_i - \widehat{Y}_i)^2} \tag{6}$$

Mean absolute error (MAE) computes the average difference between predicted and observed values, and low scores reflect the better performance of the model.

$$MAE = \frac{1}{n}\sum_{i=1}^{n}\left|Y_i - \widehat{Y}_i\right| \tag{7}$$

Mean absolute percentage error (MAPE) is a prediction error in percentage, and high scores lead to poor model performance and vice versa.

$$MAPE = \frac{1}{n}\sum_{i=1}^{n}\left|\frac{Y_i - \widehat{Y}_i}{Y_i}\right| \times 100 \tag{8}$$

Mean squared logarithmic error (MSLE) computes the logarithmic scale of errors and is suitable for datasets with exponential relationships.

$$MSLE = \frac{1}{n}\sum_{i=1}^{n}\left(\log\left(Y_i + 1\right) - \log\left(\widehat{Y}_i + 1\right)\right)^2 \quad (9)$$

Median absolute error (MedAE) is the median of absolute differences, and it reduces the impact of outliers in error evaluation.

$$MedAE = median\left(\left|Y_1 - \widehat{Y}_1\right|, \ldots, \left|Y_n - \widehat{Y}_n\right|\right) \quad (10)$$

Y_i = measured value; $\widehat{Y}_i$ = predicted value; $\overline{Y}$ = measured value's mean; n = count of samples.

Results

Shoot formation started within one week of culture containing different concentrations of *J. rubens* and *C. barbata* extract, and after 4 weeks, the shoots completely covered the petri dishes (Fig. 1a–l). Therefore, measurements of shoot clump diameter, rooting, clump fresh weight, clump dry weight and chlorophyll analyses were performed 4 weeks after culture initiation. MS media with varying concentrations and without seaweed extract were used as controls for the tested extract ratios.

Effect of seaweed extracts on micropropagation of *Micranthemum tweediei*

Addition of *J. rubens* extracts to nutrient media significantly increased the micropropagation of *M. tweediei* compared to the control MS media without extract (Figs. 1 and 2 and Table 1). The highest clump diameter (5.18 cm) was recorded on 2 g L^{-1} MS medium supplemented with 5.0% extract, which was 47% higher than the result obtained (3.52 cm) from the control 2 g L^{-1} MS without extract (Figs. 1a, c and 2a and Table 1). However, a statistically similar result was also attained from other combinations of 2.5, 5.0, and 10.0% extract with 1.0 g L^{-1} MS, or 2.5% and 10.0% extract with 2.0 g L^{-1} MS (Fig. 1a, d). It was also noted that 1.0 g L^{-1} MS with low extract concentration (2.5%) was sufficient to promote shoot proliferation, whereas supplementation of 10.0% and 20.0% extract exerted a negative impact and resulted in reduced clump diameter (Table 1, Figs. 1e and 2a). Maximum rooting rate (92.4%) and fresh weight (3.60 g) were also recorded from the supplementation of 5.0% extract and 2.0 g L^{-1} MS with increases of 173% and 108%, respectively, compared to the control 2 g L^{-1} MS without extract (Figs. 1f and 2b, c). However, supplementation of 10.0% extract and 2 g L^{-1} MS was statistically similar for rooting rate (89.3%) and clump fresh weight (Table 1). On the contrary, the highest dry clump weight (0.22 g) was obtained from 5.0% extract and 1 g L^{-1} MS (Fig. 2c). Results of shoot proliferation and rooting from *J. rubens* extract revealed that the combination of 5.0% extract and 2 g L^{-1} MS is well sufficient for enhancing clump diameter and rooting, whereas dry weight was regulated by MS concentration, and low MS concentration was found suitable for biomass production (Table 1). Overall results revealed the need for relatively low MS than the standard rate of MS used in the medium, possibly due to nutrients provided by *J. rubens*.

The addition of *C. barbata* extract to MS nutrient medium also significantly increased the in vitro micropropagation of *M. tweediei* compared to control MS media without seaweed extract (Figs. 1 and 3, Table 2). Highest clump diameter (5.11 cm), rooting rate

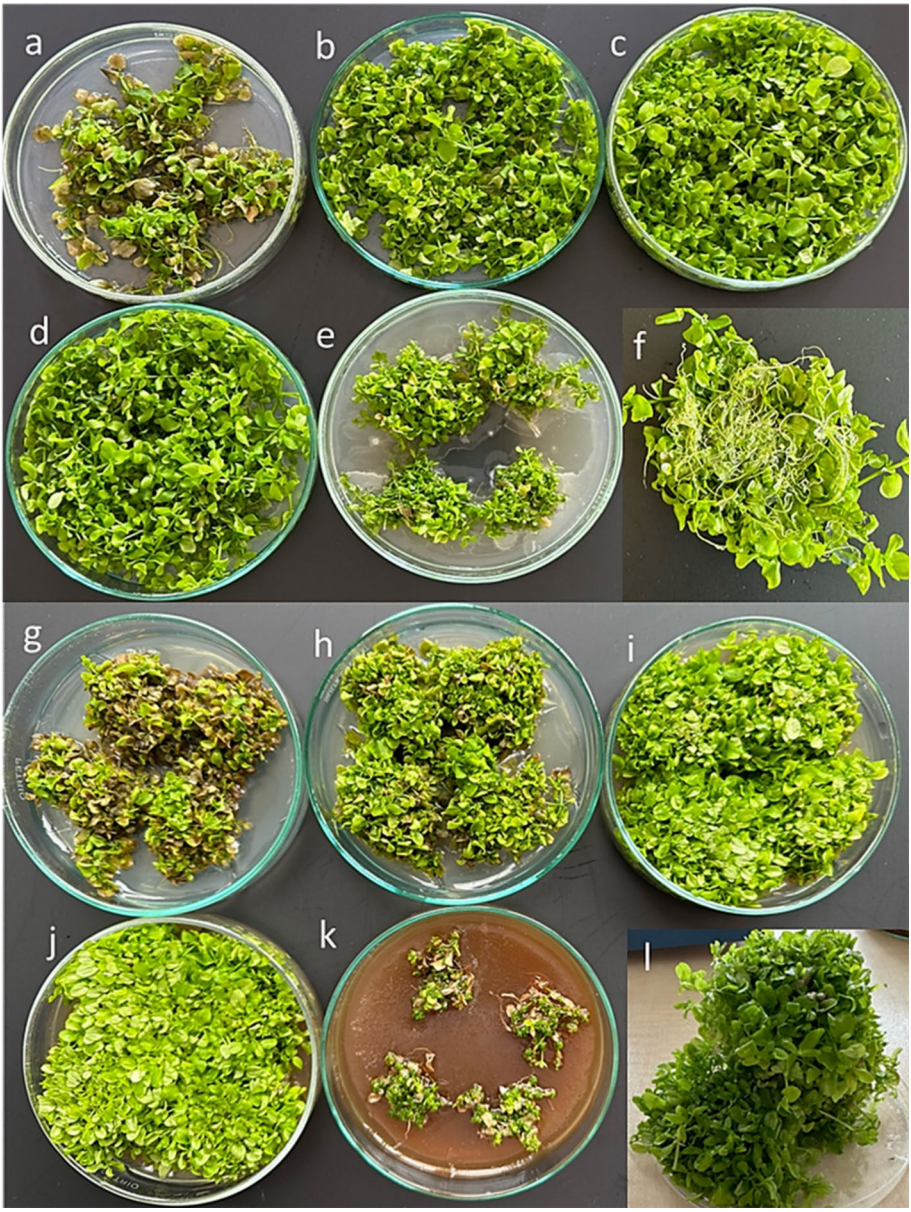


Fig. 1 Effect of extracts from *Jania rubens* and *Cystoseira barbata* seaweeds on micropropagation of *Micranthemum tweediei*. *M. tweediei* clumps growing on 2 g L^{-1} MS medium containing no (**a**), 2.5% (**b**), 5% (**c**), 10% (**d**), and 20% (**e**) of *J. rubens* extracts. **f** Rooted *M. tweediei* clump developed on 2 g L^{-1} MS with 5% *J. rubens* extract. *M. tweediei* clumps obtained on 4 g L^{-1} MS medium containing no (**g**), 2.5% (**h**), 5% (**i**), 10% (**j**), and 20% (**k**) of *C. barbata* extracts. **l** *M. tweediei* clump developed on 4 g L^{-1} MS with 10% *C. barbata* extract

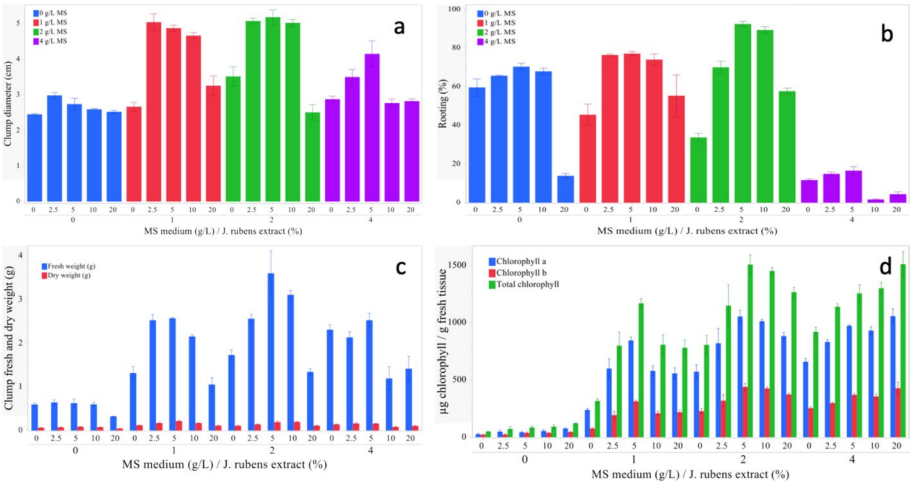


Fig. 2 Effect of *Jania rubens* extracts added to MS medium at different concentrations on micropropagation and chlorophyll content of *Micranthemum tweediei*. Clump diameter (**a**), rooting rate (**b**), clump fresh and dry weight (**c**), chlorophyll content (**d**). Bars show the standard error (SE) of the means

Table 1 Effect of *Jania rubens* extracts added to MS medium at different concentrations on micropropagation of *Micranthemum tweediei*

MS medium (g L^{-1})	Seaweed extract (%)	Clump diameter (cm) ± SE	Rooting rate (%) ± SE	Clump fresh weight (g) ± SE	Clump dry weight (g) ± SE
0	0	$2.45 \pm 0.0.2^{f}$	59.5 ± 4.41^{defg}	0.59 ± 0.03^{gh}	$0.06 \pm 3e\text{-}3^{fg}$
0	2.5	2.98 ± 0.09^{def}	$65.7 \pm 0.36c^{def}$	0.64 ± 0.06^{gh}	$0.07 \pm 3e\text{-}3^{fg}$
0	5	2.74 ± 0.16^{def}	70.3 ± 1.76^{cdef}	0.63 ± 0.09^{gh}	0.09 ± 0.01^{efg}
0	10	2.59 ± 0.02^{ef}	67.9 ± 1.61^{cdef}	0.60 ± 0.05^{gh}	$0.08 \pm 3e\text{-}3^{efg}$
0	20	2.52 ± 0.04^{f}	13.9 ± 1.2^{j}	0.32 ± 0.01^{h}	$0.04 \pm 3e\text{-}3^{g}$
1	0	2.67 ± 0.12^{def}	45.5 ± 5.5^{gh}	1.32 ± 0.15^{efg}	0.12 ± 0.01^{cdef}
1	2.5	5.04 ± 0.23^{ab}	76.4 ± 0.49^{abcd}	2.52 ± 0.13^{bc}	0.17 ± 0.02^{abc}
1	5	4.87 ± 0.08^{ab}	77.1 ± 1.08^{abc}	2.57 ± 0.02^{bc}	0.22 ± 0.02^{a}
1	10	4.66 ± 0.09^{ab}	74.0 ± 2.9^{bcde}	2.15 ± 0.04^{cde}	0.17 ± 0.01^{abc}
1	20	3.26 ± 0.27^{cdef}	55.3 ± 10.8^{fg}	1.05 ± 0.15^{fgh}	0.11 ± 0.02^{cdef}
2	0	3.52 ± 0.27^{cd}	33.8 ± 2.1^{hi}	1.73 ± 0.12^{cdef}	0.11 ± 0.01^{cdef}
2	2.5	5.07 ± 0.08^{a}	70 ± 3.19^{cdef}	$2.56 \pm 0.1b^{c}$	0.14 ± 0.01^{bcde}
2	5	5.18 ± 0.20^{a}	92.4 ± 1.39^{a}	3.60 ± 0.51^{a}	0.19 ± 0.02^{ab}
2	10	5.02 ± 0.10^{ab}	89.3 ± 1.74^{ab}	3.11 ± 0.10^{ab}	0.20 ± 0.01^{ab}
2	20	2.51 ± 0.21^{f}	57.7 ± 1.59^{efg}	1.34 ± 0.07^{efg}	0.11 ± 0.01^{cdef}
4	0	$2.88 \pm 0.09d^{ef}$	11.7 ± 0.65^{j}	2.31 ± 0.12^{bcd}	0.14 ± 0.01^{bcde}
4	2.5	3.50 ± 0.21^{cde}	14.9 ± 1.02^{j}	2.13 ± 0.13^{cde}	0.16 ± 0.02^{abcd}
4	5	4.15 ± 0.37^{bc}	16.6 ± 2.06^{ij}	2.53 ± 0.16^{bc}	0.15 ± 0.01^{abcd}
4	10	2.77 ± 0.11^{def}	1.7 ± 0.33^{j}	1.19 ± 0.27^{fgh}	0.08 ± 0.01^{efg}
4	20	2.82 ± 0.06^{def}	4.4 ± 1.16^{j}	1.42 ± 0.28^{defg}	0.10 ± 0.01^{defg}

The difference between the means shown in different letters in the same column is significant at the 0.05 level according to the Tukey test

(42.8%), clump fresh weight (7.37 g), and clump dry weight (0.59 g) were obtained from 4 g L^{-1} MS with 10% *C. barbata* extract (Figs. 1j, 1 and 3a–c and Table 2). These results were 81%, 113%, and 436% higher than the control 4 g L^{-1} MS medium without extract, respectively. Supplementation of 5.0% extract and 4 g L^{-1} MS also enhanced the shoot proliferation better than control (Fig. 1g, i), whereas 20 g L^{-1} *C. barbata* extract with 2 and 4 g L^{-1} MS resulted in reduced clump diameter and rooting rate (Fig. 1k, Table 2).

When comparing the effects of *J. rubens* and *C. barbata* on clump diameter and rooting rate of shoots in *M. tweediei*, lower MS usage yielded higher results with lower *J. rubens* extract. *J. rubens* achieved the highest clump diameter (5.18 cm) and rooting rate (92.4%) from 2 g L^{-1} MS with 5% extract, while *C. barbata* resulted in the highest clump diameter (5.11 cm) and rooting rate (42.8%) in 4 g L^{-1} MS with 10% extract (Figs. 2 and 3, Tables 1 and 2). However, clump fresh weight and dry weight were found to be higher in 4 g L^{-1} MS medium containing 10% *C. barbata* extract than in MS media containing *J. rubens* extract (Figs. 2 and 3, Table 3).

Effect of seaweed extracts on chlorophyll contents of *M. tweediei*

Supplementation of *J. rubens* and *C. barbata* extracts to MS media resulted in greener shoot clumps than control media without extract and significant impact on all pigments (chlorophyll *a*, chlorophyll *b*, and total chlorophyll) in both seaweeds (Table 3, Figs. 2d and 3d). Use of 20.0% *J. rubens* extract and 4 g L^{-1} MS yielded highest amounts of chlorophyll *a* (1057 µg chlorophyll g fresh tissue^{-1}) and total chlorophyll (1511 µg chlorophyll g fresh tissue^{-1}). However, it was found statistically similar results with 5.0% *J. rubens* extract and 2 g L^{-1} MS and yielded the highest amount of chlorophyll *b* (439 µg chlorophyll g fresh tissue^{-1}) (Table 3, Fig. 2d). In control MS media without extract, chlorophyll *a* (658 µg chlorophyll g fresh tissue^{-1}), chlorophyll *b* (228 µg chlorophyll g fresh tissue^{-1}), and total chlorophyll (920 µg chlorophyll g fresh tissue^{-1}) values were found to be much lower. Addition of 5.0% *C. barbata* extract to 4 g L^{-1} MS resulted in highest chlorophyll *a* (811 µg chlorophyll g fresh tissue^{-1}) and total

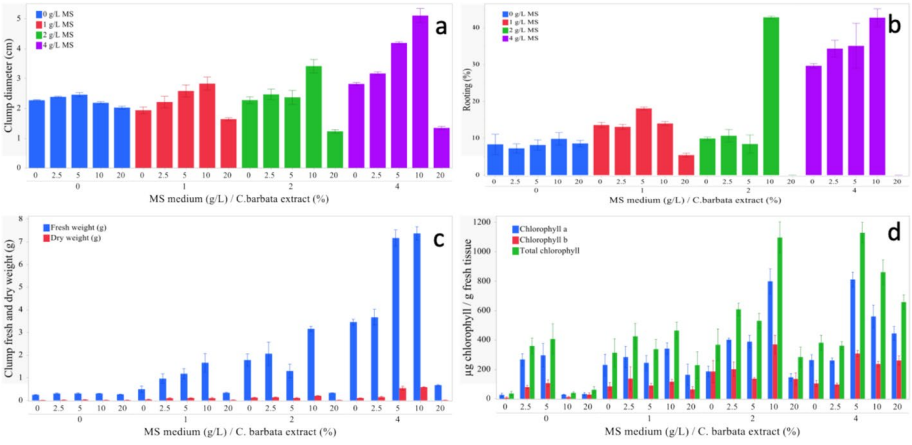


Fig. 3 Effect of *Cystoseira barbata* extracts added to MS medium at different concentrations on micropropagation and chlorophyll content of *Micranthemum tweediei*. Clump diameter (**a**), rooting rate (**b**), clump fresh and dry weight (**c**), chlorophyll content (**d**). Bars show the standard error (SE) of the means

Table 2 Effect of *Cystoseira barbata* extracts added to MS medium at different concentrations on micropropagation of *Micranthemum tweediei*

MS medium (g L^{-1})	Seaweed extract (%)	Clump diameter (cm) ± SE	Rooting rate (%) ± SE	Clump fresh weight (g) ± SE	Clump dry weight (g) ± SE
0	0	2.28 ± 0.01^{efg}	8.3 ± 4.77^{cde}	0.25 ± 0.03^{g}	0.02 ± 0.001^{c}
0	2.5	2.39 ± 0.02^{ef}	7.2 ± 2.17^{de}	0.30 ± 0.04^{g}	0.02 ± 0.004^{c}
0	5	2.46 ± 0.07^{def}	8.2 ± 2.4^{cde}	0.31 ± 0.05^{g}	0.03 ± 0.009^{c}
0	10	2.19 ± 0.04^{efg}	9.8 ± 2.98^{cde}	0.31 ± 0.02^{g}	0.02 ± 0.001^{c}
0	20	2.03 ± 0.05^{fgh}	8.6 ± 1.44^{cde}	0.27 ± 0.03^{g}	0.02 ± 0.002^{c}
1	0	1.94 ± 0.11^{fghı}	13.7 ± 1.26^{cd}	0.49 ± 0.23^{fg}	0.04 ± 0.019^{c}
1	2.5	2.22 ± 0.19^{efg}	13.1 ± 1.15^{cd}	0.96 ± 0.36^{defg}	0.10 ± 0.026^{bc}
1	5	2.59 ± 0.20^{def}	18.1 ± 0.72^{c}	1.18 ± 0.39^{defg}	0.11 ± 0.023^{bc}
1	10	2.83 ± 0.22^{cde}	14.0 ± 0.9^{cd}	1.66 ± 0.71^{def}	0.10 ± 0.035^{bc}
1	20	1.64 ± 0.05^{ghı}	5.4 ± 0.95^{de}	0.34 ± 0.03^{g}	0.02 ± 0.003^{c}
2	0	2.28 ± 0.11^{efg}	9.9 ± 0.76^{cde}	1.78 ± 0.49^{de}	0.13 ± 0.012^{bc}
2	2.5	2.47 ± 0.18^{def}	10.7 ± 2.96^{cd}	2.06 ± 0.88^{cd}	0.14 ± 0.006^{bc}
2	5	2.37 ± 0.23^{ef}	8.4 ± 4.3^{cde}	1.30 ± 0.53^{defg}	0.11 ± 0.025^{bc}
2	10	3.42 ± 0.22^{c}	42.8 ± 0.63^{a}	3.16 ± 0.20^{bc}	0.21 ± 0.009^{b}
2	20	1.23 ± 0.06^{ı}	0.0 ± 0.00^{e}	0.33 ± 0.01^{g}	0.02 ± 0.00^{c}
4	0	2.82 ± 0.05^{cde}	29.7 ± 1.04^{b}	3.46 ± 0.22^{b}	0.11 ± 0.007^{bc}
4	2.5	3.17 ± 0.05^{cd}	34.3 ± 3.96^{ab}	3.67 ± 0.61^{b}	0.15 ± 0.04^{bc}
4	5	4.20 ± 0.04^{b}	35.1 ± 10.6^{ab}	7.17 ± 0.62^{a}	0.54 ± 0.082^{a}
4	10	5.11 ± 0.24^{a}	42.8 ± 4.16^{a}	7.37 ± 0.51^{a}	0.59 ± 0.015^{a}
4	20	1.35 ± 0.06^{hı}	0.0 ± 0^{e}	0.68 ± 0.0^{6efg}	0.02 ± 0.002^{c}

The difference between the means shown in different letters in the same column is significant at the 0.05 level according to the Tukey test

chlorophyll (1129 µg chlorophyll g fresh $tissue^{-1}$) amounts, whereas the highest chlorophyll *b* (369 µg chlorophyll g fresh $tissue^{-1}$) was recorded from 10.0% *C. barbata* extract and 2.0 g L^{-1} MS (Table 3, Fig. 3d). These results were higher than the chlorophyll *a* (263 µg chlorophyll g fresh $tissue^{-1}$), chlorophyll *b* (186 µg chlorophyll g fresh $tissue^{-1}$), and total chlorophyll (381 µg chlorophyll g fresh $tissue^{-1}$) levels obtained from control MS media without extract. Considering all the results regarding the chlorophyll content of *M. tweediei*, the addition of both seaweed extracts to the nutrient media significantly increased the levels of chlorophyll *a*, chlorophyll *b*, and total chlorophyll compared to the control media without extract.

Response surface regression analysis (RSRA)

Results of response surface regression analysis are presented in Table S and revealed the statistically significant impact of model and linear analysis for all output parameters. In general, individual input variables or their interactions were statistically significant, with a non-significant impact recorded for some input variables. Input variables like conc., algae, conc.×algae, and conc.×MS had no impact on dry weight. Interaction of conc.×algae and conc.×MS also exerted no impact on clump diameter and rooting. All chlorophyll

Table 3 Effect of *Jania rubens* and *Cystoseira barbata* extracts added to MS medium at different concentrations on chlorophyll content of *Micranthemum tweediei*

MS ($g\ L^{-1}$)	Seaweed extract (%)	*Jania rubens* (µg chlorophyll g fresh tissue^{-1}) ± SE			Cystoseira barbata (µg chlorophyll g fresh tissue^{-1}) ± SE	*Cystoseira barbata* (µg chlorophyll g fresh tissue^{-1}) ± SE	
		Chlorophyll *a*	Chlorophyll b	Total chlorophyll	Chlorophyll *a*	Chlorophyll b	Total chlorophyll
0	0	27 ± 7.26^{f}	21 ± 5.44^{h}	48 ± 2.67^{e}	27 ± 11.37^{e}	7 ± 5.18^{f}	37 ± 15.16 g
0	2.5	49 ± 13.1^{f}	23 ± 5.79^{h}	72 ± 18.7^{e}	268 ± 39.2^{cde}	79 ± 11.65^{cdef}	359 ± 53.23cdefg
0	5	43 ± 6.55^{f}	37 ± 7.36^{h}	84 ± 10.8^{e}	296 ± 79.16^{bcde}	107 ± 22.65^{cdef}	407 ± 102.6cdefg
0	10	54 ± 12^{f}	37 ± 6.08^{h}	92 ± ± 16.7^{e}	28 ± 4.169^{e}	12 ± 4.17^{f}	40 ± 7.528 g
0	20	75. ± 3.9^{f}	45 ± 3.44^{h}	121 ± 2.79^{e}	32 ± 10.09^{e}	29 ± 11.47^{ef}	62 ± 21.67cdefg
1	0	238 ± 11.5^{f}	76 ± 10.1^{h}	316 ± 20.1^{e}	231 ± 72.36^{cde}	84 ± 26.02^{cdef}	314 ± 95.06cdefg
1	2.5	599 ± 84.3^{cde}	193 ± 32.9^{fg}	799 ± 118^{cd}	284 ± 71.95^{cde}	138 ± 79.68^{bcdef}	425 ± 87.72cdef
1	5	845 ± 30.1^{abc}	313 ± 10.3^{bcde}	1168 ± 40.8^{abc}	245 ± 49.84^{cde}	90 ± 15.13^{cdef}	338 ± 65.56cdefg
1	10	579 ± 43.8^{de}	209 ± 17.3^{ef}	808 ± 86.6^{cd}	342 ± 39.09^{bcd}	116 ± 15.75^{cdef}	464 ± 57.53cde
1	20	557 ± 49.2^{e}	218 ± 16.6^{ef}	781 ± 66.2^{d}	163 ± 72.25^{de}	64 ± 18.6^{def}	229 ± 91.21cdefg
2	0	572 ± 59de	228 ± 21.8^{ef}	807 ± 80.9^{cd}	186 ± 36.15^{cde}	186 ± 75.38^{abcdef}	368 ± 107.7cdefg
2	2.5	820 ± 129^{abcd}	319 ± 51.9^{bcde}	1148 ± 182^{abcd}	402 ± 10.88^{bcd}	201 ± 50.18^{abcde}	609 ± 41.29bcd
2	5	1054 ± 54.4^{a}	439 ± 29.1^{a}	1506 ± 84.3^{a}	389 ± 42.33^{bcd}	137 ± 8.79^{bcdef}	530 ± 51.48bcde
2	10	1012 ± 17.2^{a}	426 ± 12.1^{ab}	1451 ± 29.9^{a}	799 ± 84.59^{a}	369 ± 62.85^{a}	1097 ± 106.3a
2	20	883 ± 30.4^{ab}	373 ± 11.6^{abc}	1266 ± 41.7^{ab}	146 ± 25.5^{de}	135 ± 41.97^{bcdef}	284 ± 67.44cdefg
4	0	658 ± 28.3^{bcde}	253 ± 11.7^{def}	920 ± 39.5^{bcd}	263 ± 36.15^{cde}	105 ± 20.49^{cdef}	381 ± 51.39cdefg
4	2.5	831 ± 20.1^{abc}	297 ± 7.47^{cdef}	1139 ± 26.4^{abcd}	261 ± 16.97^{cde}	98 ± 10.22^{cdef}	361 ± 26.96cdefg
4	5	972 ± 9.91^{a}	369 ± 13^{abcd}	1256 ± 74.8^{ab}	811 ± 49.21^{a}	308 ± 20.45^{ab}	1129 ± 70.43^{a}
4	10	931 ± 33.7^{a}	355 ± 19.1^{abcd}	1300 ± 51.1^{ab}	560 ± 76.9^{ab}	237 ± 17.07^{abcd}	861 ± 83.25ab
4	20	1057 ± 65^{a}	428 ± 53.1^{ab}	1511 ± 111^{a}	445 ± 46.89^{bc}	261 ± 30.8^{abc}	658 ± 49.14bc

The difference between the means shown in different letters in the same column is significant at the 0.05 level according to the Tukey test

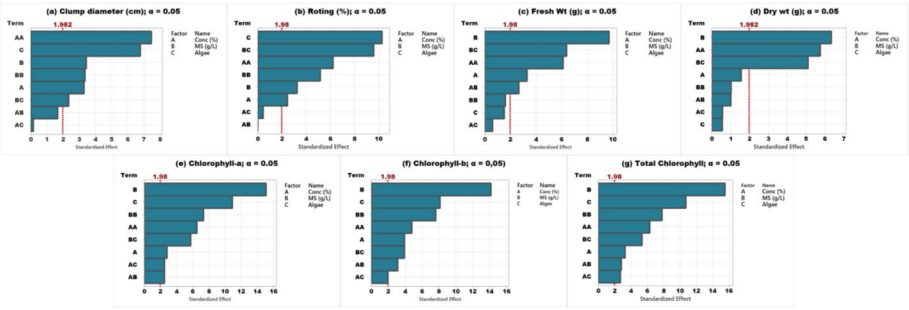


Fig. 4 Pareto chart analysis of seaweed extracts on in vitro organogenesis, biomass, and pigmentation of *Micranthemum tweediei*. **a** clump diameter, **b** rooting, **c** fresh weight, **d** dry weight, **e** chlorophyll *a*, **f** chlorophyll *b*, and **g** total chlorophyll

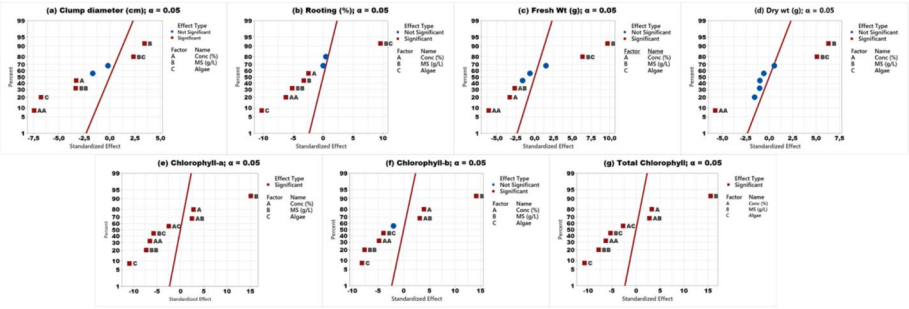


Fig. 5 Normal plot analysis of seaweed extracts on in vitro organogenesis, biomass, and pigmentation of *Micranthemum tweediei*. **a** Clump diameter, **b** rooting rate, **c** fresh weight, **d** dry weight, **e** chlorophyll *a*, **f** chlorophyll *b*, and **g** total chlorophyll

parameters were found significant in response to all input variables. Results were further confirmed by a Pareto chart that revealed the significance pattern of AA-C-B-BB-A-BC-AB-AC for clump diameter (Fig. 4a), C-BC-AA-BB-B-A-AC-AB for rooting (Fig. 4b), B-BC-AA-A-AB-BB-C-AC for fresh weight (Fig. 4c), B-AA-BC-A-BB-AA-AC-C for dry weight (Fig. 4d), B-C-BB-AA-BC-A-AC-BC for chlorophyll *a* (Fig. 4e), B-C-BB-AA-A-BC-AB-AC for chlorophyll *b* (Fig. 4f), and B-C-BB-AA-BC-A-AB-AC for total chlorophyll (Fig. 4g). It was observed that MS was the most significant factor for all parameters, except clump diameter and rooting, whereas AC was the least significant factor for clump diameter, fresh weight, chlorophyll *b*, and total chlorophyll. In general, a minor difference was observed between fresh weight and dry weight and all chlorophyll contents. In the next step, the significance of input factors was evaluated by normal plot analysis (Fig. 5a–g), which revealed the positive correlation between MS and other parameters and confirmed the Pareto chart analysis. BC was the other factor that was significant and exhibited a positive correlation with clump diameter, rooting, and biomass. Algae type exerted a negative impact on clump diameter, rooting, and all chlorophyll contents. However, the concentration of algae extracts in the medium resulted in enhanced chlorophyll contents but reduced the biomass and rooting.

Response surface regression analysis (RSRA) of individual seaweed

Results of RSRA analysis of combined input factors revealed the significance of seaweed, and their individual impact was also computed to measure the efficacy and impact of in vitro shoot proliferation and chlorophyll contents. Results of *J. rubens* revealed the significance pattern of BB-AA-A-B-AB for clump diameter, BB-B-AA-AB-A for rooting, BB-B-AA-A-AB for fresh weight, BB-AA-B-A-AB for dry weight, B-BB-AA-A-AB for chlorophyll *a*, and B-BB-A-AA-AB for chlorophyll *b* and total chlorophyll contents (Fig. S1a–g). Results of normal plot analysis of *J. rubens* revealed the negative impact of increased extract concentration on clump diameter. Increased MS concentration illustrated the negative impact on rooting, while enhanced rooting was attributed to conc × MS. A similar pattern was recorded for both fresh weight and dry weight, with a positive correlation of MS and a negative correlation of seaweed extract concentration for both parameters. In contrast, a positive correlation between seaweed conc., MS, and conc. × MS was observed for all chlorophyll contents (Fig. S2a–g). Results of response optimization confirmed the findings, with seaweed concentration of 10.10 mg L^{-1} and 2.67 g L^{-1} MS optimized for attaining maximum shoot proliferation and chlorophyll contents (Table 4).

Results of the Pareto chart for *C. barbata* extract exhibited a different pattern and were recorded as AA-B-A-BB-AA for fresh weight, AA-B-AB-A-BB for rooting, B-AA-AB-A-BB for fresh weight, B-AA-A-AB-BB for dry weight, B-AA-AB-BB-A for chlorophyll *a*, and B-AA-BB-AB-A for chlorophyll *b* and total chlorophyll (Fig. S3a–g). Results of normal plots revealed a similar pattern, and the impact of MS was positive, with all parameters increasing with MS concentration, whereas the increase of *C. barbata* extract's concentration resulted in decreased biomass and chlorophyll contents (Fig. S4a–g). Results were confirmed by response optimization that optimized the supplementation of 9.90 mg L^{-1} *C. barbata* extract with 4.0 g L^{-1} MS for obtaining maximum biomass and chlorophyll contents (Table 4).

Response optimization

Results of response optimization of both seaweeds are presented in Table 4. For response optimization, all output parameters were adjusted to maximum, except rooting (do not optimize). Results revealed the almost similar concentration of extracts for both seaweeds but with different MS concentrations. Supplementation of 2.0 and 4.0 g L^{-1} MS was found optimal for yielding maximum values of all responses for *J. rubens* and *C. barbata*, respectively. It was also noted that biomass (fresh and dry weight) was maximum from *C. barbata*, while all other parameters were optimized higher for *J. rubens* seaweed (Table 4).

Machine learning analysis

The results of ML analysis of three models based on six performance metrics of three models are presented in Table 5. Results of R^2 scores illustrated the highest score from RF–MLP–XGB for clump diameter and dry weight, MLP–RF–XGB for rooting, fresh weight, and chlorophyll *a*. The order of RF–XGB–MLP and RF = MLP–XGB was recorded for chlorophyll *b* and total chlorophyll. Results were further confirmed by RMSE, which revealed the lowest RMSE score for rooting, fresh weight, and chlorophyll *a*. The lowest

Table 4 Response optimization of *Jania rubens* and *Cystoseira barbata* extracts on in vitro organogenesis. Biomass and pigments of *Micranthemum tweediei*

Solution	Conc (%)	MS (g L^{-1})	Total chlorophyll	Chlorophyll *b*	Chlorophyll *a*	Dry weight (g)	Fresh weight (g)	Clump diameter (cm)	Composite desirability
J. rubens	10.101	2.67	1559.16	438.00	1113.01	0.187	2.884	4.879	0.785
C. barbata	9.899	4	909.69	263.26	640.93	0.373	5.326	3.928	0.637

Table 5 Performance metrics for the ML models for in vitro organogenesis. Biomass and pigments of *Micranthemum tweediei*

	R^2	RMSE	MAE	MAPE	MLSE	MedAE
RF						
Clump diameter (cm)	0.890	0.364	0.271	0.09437	0.008	0.179
Rooting rate (%)	0.865	10.419	6.818		0.241	3.844
Fresh weight (g)	0.921	0.464	0.336	0.28821	0.027	0.224
Dry weight (g)	0.894	0.039	0.027	0.35895	0.001	0.020
Chlorophyll-a	0.870	122.721	88.957	0.39301	0.188	62.588
Chlorophyll-b	0.800	61.434	42.395	0.67641	0.330	31.090
Total chlorophyll	0.872	168.268	125.744	0.35369	0.161	101.978
XGBoost						
Clump diameter (cm)	0.888	0.367	0.273	0.09736	0.009	0.164
Rooting rate (%)	0.859	10.660	6.485		0.212	3.458
Fresh weight (g)	0.920	0.466	0.331	0.26959	0.026	0.211
Dry weight (g)	0.868	0.043	0.029	0.34325	0.001	0.020
Chlorophyll *a*	0.869	123.215	89.440	0.35054	0.181	68.607
Chlorophyll *b*	0.798	61.614	43.871	0.51071	0.280	29.307
Total chlorophyll	0.869	170.680	128.526	0.35432	0.240	91.188
MLP						
Clump diameter (cm)	0.889	0.367	0.266	0.09322	0.008	0.174
Rooting rate (%)	0.869	10.268	6.223		0.187	2.994
Fresh weight (g)	0.926	0.452	0.322	0.2663	0.026	0.199
Dry weight (g)	0.893	0.039	0.026	0.32544	0.001	0.019
Chlorophyll *a*	0.871	122.311	89.503	0.34481	0.165	62.930
Chlorophyll *b*	0.796	61.938	44.279	0.53717	0.271	30.101
Total chlorophyll	0.872	168.429	126.738	0.31402	0.142	99.876

RMSE for clump diameter, dry weight, chlorophyll *b*, and total chlorophyll was recorded from the RF model. XGBoost ranked third for all parameters. Results of MAE illustrated the lowest MAE for clump diameter, rooting rate (%), fresh weight, and dry weight by the MLP model, whereas all chlorophyll parameters had the lowest MAE from the RF model. The performance of the XGBoost model was comparatively low as ranked third for most of the parameters. Investigation of MAPE exhibited the lowest relative error for clump diameter, fresh weight, dry weight, and total chlorophyll by the MLP model, while the XGBoost model performed better than RF for chlorophyll *a* and chlorophyll *b* contents. Results of MLSE revealed variable responses: the lowest values of rooting, chlorophyll *a*, and total chlorophyll were attributed to the MLP model, while the RF model performed better for clump diameter and dry weight. In contrast, the lowest MLSE score of fresh weight and chlorophyll *b* was recorded by the XGBoost model. Results associated with MedAE revealed that the MLP model had the lowest score for rooting, fresh weight, and dry weight. Conversely, the lowest MedAE scores of clump diameter, chlorophyll *b*, and total chlorophyll were recorded by the XGBoost model, while the RF model excelled in the minimum MedAE score for chlorophyll *a*. Comparison of models revealed the better performance of the MLP model due to high R^2 and relatively lower metrics for morphological traits. On the contrary, the RF model exhibited better performance for biochemical traits.

The XGBoost model predicted the performance of chlorophyll *b* model more precisely compared to other morphological and chlorophyll traits. The graphical presentation of ML results is presented in Fig. 6a – g, which illustrated the inclination of predicted scores close to the 1:1 line for models with high R^2 scores.

Discussion

In vitro nutrient media are one of the most important factors affecting the rapid and effective micropropagation of plants (Özcan et al. 2023; Özcan and Atar 2025), especially for recalcitrant plants (Yaqoob et al. 2021; Boboc Oros et al. 2022). Moreover, the addition of biostimulants to the nutrient media is inevitable for morphogenesis. Underutilized seaweeds are an important bioresource for food, agriculture, industry, and therapeutics and also have important bio-stimulant properties that enhance plant growth when used in small amounts (Anbazhakan et al. 2022). The amount and composition of bio-stimulants in seaweeds vary with species and environmental conditions (Kadam et al. 2015; Anbazhakan et al. 2022; Mughunth et al. 2024). Most seaweed extracts contain organic minerals, plant growth regulators such as GA_3, IAA, and kinetin, and

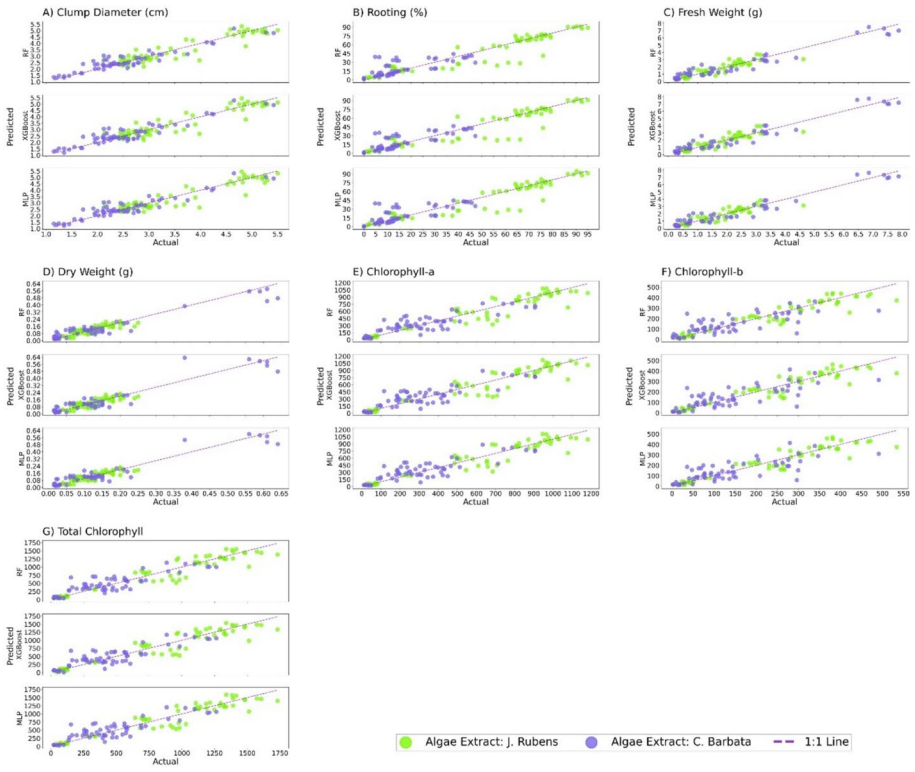


Fig. 6 Machine learning analysis of seaweed extracts on in vitro organogenesis, biomass, and pigmentation of *Micranthemum tweediei* **a** clump diameter, **b** rooting rate, **c** fresh weight, **d** dry weight, **e** chlorophyll *a*, **f** chlorophyll *b*, and **g** total chlorophyll

thus, they stimulate growth and development in many plant species (Calvo et al. 2014; Kakade et al. 2022). Transcriptomic analyses have also shown that seaweed extracts upregulated many genes related to hormone metabolism and plant development (Jannin et al. 2013; Goni et al. 2016). Therefore, naturally occurring seaweeds have a great potential as inexpensive biostimulants for plant growth.

Like other seaweed species, *J. rubens* and *C. barbata* have also important bio-stimulant properties with organic minerals and plant growth regulators (Hamamouche et al. 2024; Mutlu-Durak et al. 2024). In the present study, *J. rubens* and *C. barbata* extracts, which are abundant in the sea and oceans, were used for the first time on in vitro micropropagation of ground macrophyte *M. tweediei*, an important aquarium/aquatic plant. The addition of seaweed extract to the nutrient media significantly increased in vitro plant regeneration and the chlorophyll levels in regenerating plants compared to the control media without extract. Considering the shoot clump diameter, rooting rate, and clump fresh and dry weight, the highest rate of micropropagation in *M. tweediei* was obtained from 2 g L^{-1} MS medium containing 5% *J. rubens* extract. On the contrary, a relatively high concentration of *C. barbata* extract with 4 g L^{-1} MS was found efficient for shoot proliferation. The main reason why *J. rubens* extract provides higher micropropagation at lower MS concentrations compared to *C. barbata* extract may be due to the variation in organic minerals, biostimulants, and phenolics in both species (Manev et al. 2013; Khairy and El-Shafay 2013; Hamamouche et al. 2024; Mutlu-Durak et al. 2024; Hawas et al. 2025; Özcan and Atar 2025). By adding seaweed extracts to nutrient media, there was also no need for plant growth regulators, which are essential compounds of nutrient media, and using half of the recommended amount of media with 2.5 or 5% *J. rubens* extract was sufficient.

Results also revealed the negative impact of high concentration (20%) of both seaweed extract that significantly reduced shoot regeneration and rooting. Similarly, in our previous study, the highest micropropagation in *Anubias barteri* and *Alternanthera reineckii* was obtained when 2.5 g L^{-1} J. *rubens* and *C. barbata* extracts were added to 4 g L^{-1} MS medium. In contrast, 20% *J. rubens* and *C. barbata* extract in 4 g L^{-1} MS significantly reduced shoot regeneration (Özcan and Atar 2025). Previous studies also revealed the highest regeneration at low concentration of 4% *Sargassum ilicifolium* extract in *Plumbago zeylanica,* while the shoots showed the best rooting in the medium supplemented with 2.5% extract (Kakade et al. 2022). In the same study, the addition of 10% extract to the nutrient medium significantly reduced shoot regeneration. Our results are also in line with the previous work documented on *Solanum melongena*, and the highest regeneration was attained from ½ MS media containing 3% or 4% extract of *Gracilaria salicornia*, *Padina gymnospora*, *Padina boergesenii*, and *Gelidiella acerosa* (Satish et al. 2015). Similar results were also obtained from in vitro growth of *Nicotiana benthamiana* by using *Cystoseira myriophylloides*, *Fucus spiralis*, and *Laminaria digitata* extracts with MS medium (Esserti et al. 2017). In the same study, it was also reported that the reason for the decrease in plant growth when high concentrations of seaweed extract are used may be due to the substantial levels of phenolic compounds in the seaweed extracts (Eserti et al. 2017). It is well known that in tissue cultures of plants containing high levels of phenolic compounds, phenolics secreted from wounded regions of explants cause browning of culture media and plant tissues and inhibition of growth as a result of polyphenol oxidases (Ndakidemi et al. 2014; Irshad et al. 2018; Amente and Chimdessa 2021; Gemechu and Amente 2021; Liu et al. 2024). In the present study, using 20% *J. rubens* and *C. barbata* extracts, which were reported to contain substantial levels of phenolic compounds (El-Din and El-Ahwany

2016; Cadar et al. 2019; Trifan et al. 2019), resulted in browning of the shoots and a significant reduction in growth (Fig. 1k).

Although there are several types of photosynthetic pigments, such as carotenoids and phycobilin, chlorophylls are the most important ones in terms of capturing sunlight (Ebrahimi et al. 2023). Chlorophylls convert solar energy into chemical energy to produce essential carbohydrate molecules (glucose) that the entire plant uses as a food source (Pareek et al. 2017). Moreover, chlorophylls, which are rich in vitamins E, A, C, K, β-carotene, essential fatty acids, and minerals such as iron, magnesium, potassium, and calcium, have been reported to have high antioxidant activity, wound healing, anti-mutagenic, anti-inflammatory, and anti-cancer properties (Leite et al. 2018; Sarkar et al. 2020). Chlorophyll *a*, which is the most abundant type of pigment in plants and green algae, is the main pigment involved in photosynthesis, while chlorophyll *b* has a critical role in harvesting light at lower light intensities (Tsuzuki et al. 2022). Total chlorophyll amount and chlorophyll *a*/*b* ratio may vary depending on the stage of development, light levels, temperature, humidity, and nutrient availability (Kitajima and Hogan 2003; Wu et al. 2021; Paraginski et al. 2025). In the current study, results also revealed that the addition of *J. rubens* and *C. barbata* extracts to the nutrient media significantly raised the chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents of the in vitro regenerated aquatic plant *M. tweediei*, as found earlier in macrophytes *Anubias barteri* and *Alternanthera reineckii* (Özcan and Atar 2025). Spraying of *Ulva rigida* and *Fucus spiralis* extracts on *Phaseolus vulgaris* grown under water deficit conditions and foliar application of *Ascophyllum nodosum* extracts on *Allium cepa* and *Callistemon viminalis* plants also provided significant increases in chlorophyll *a* and *b* contents (Mansori et al. 2015; Hidangmayum and Sharma 2017; Al-Bakar et al. 2020). Similarly, the use of commercial Macys BC28 and Sefer-100 seaweed extracts in apples increased the chlorophyll content significantly (Mousavi et al. 2024).This significant increase in chlorophyll contents can be attributed to the high nutrients and growth regulators' levels in seaweed extracts (Rashad and El-Chaghaby 2020; Anbazhakan et al. 2022; Mughunth et al. 2024).

Results of ANOVA analysis illustrated the significant level and input factors for output parameters, but they are not efficient for displaying the most significant factor. Finding the most important and least significant factor with its impact on the output parameter can be computed with modern optimization tools like response surface regression analysis (RSRA). RSRA is a powerful statistical approach of experimental design and optimization to investigate the relationships between independent and dependent variables for predictive modeling and robust designing in complex systems. Analysis of RSRA illustrated the significance of the model's performance for all output variables, suggested the fitness of the experiment for finding the correlation between input and output variables, and confirmed it by linear analysis (Özcan et al. 2023; Ali and Aasim 2024). Results further confirmed a substantial portion of variability, especially for chlorophyll content parameters, whereas linear analysis presented the dominance of MS alone or with other input factors. Among the parameters, dry weight was the weakest or non-significant output parameter from both the model and linear analysis.

After computing the significance level by RSRA, results were further analyzed by Pareto charts and normal plots that are computational tools and the backbone of regression analysis and design of experiment (Ali and Aasim 2024). Pareto charts show the importance of factors by stacking the parameters and dividing them into significant and non-significant parameters by the normal probability line, whereas normal plots validate the statistical assumptions by identifying key effects and enhance the efficiency and accuracy

of experiments. Normal plots distribute the data on the left and right sides of the normal probability line, indicating the relationship between input and output parameters. In this way, normal plots provide reliable insights and help to identify root causes of variability (Alkiayat 2021; Katırcı et al. 2024). Results of the Pareto chart analysis exhibited the significant impact of input variables, and input factors regulated the in vitro regeneration with MS as the most significant factor for biomass and chlorophyll contents, suggesting the importance of minerals supplemented by MS, whereas seaweed was most significant for clump diameter and rooting. In general, seaweed type and concentration were the least important factors in this study. Results of Pareto charts were further verified by normal plots which revealed the clear impact of MS concentration and enhanced the biomass and pigmentation with an increase in MS concentration. Likewise, seaweed concentration was also significant for pigmentation and increased proportionally with concentration. Normal plot analysis also exhibited the significance of the type of algae which exerted a negative impact on regeneration, suggesting the need for a specific type of algae for in vitro regeneration, biomass, and pigmentation.

It was evident from the results that seaweed extract type, their concentration, and MS concentration were highly significant, but the clear impact of which seaweed species is better was not clear. Therefore, RSRA was performed for both species separately, and results confirmed that *J. rubens* was better than *C. barbata. J. rubens* extract resulted in maximum clump diameter, rooting, and biomass at low concentration and with low MS concentration. Chlorophyll contents were regulated in the similar manner despite of seaweed type. Results were confirmed by optimizing the biomass and chlorophyll contents of both species, which revealed the similar need for extract but with different MS concentrations. The study presents the comparison of three different models for in vitro regeneration of *M. tweediei.* Results revealed the significance of each model for predicting individual parameters. Performance metrics are the mainstay of predicting model's performance and enable the researcher to understand and make decisions for complex biological data (Erickson and Kitamura 2021; Plevris et al. 2022).

Results revealed the better performance of the MLP model for morphological parameters compared to the RF and XGBoost models, due to their different structures. The MLP model is based on a neural network used for nonlinear and complex relationships analysis. The MLP model exhibited excellent prediction for morphological data, demonstrating the ability of the model to handle and explain variance in the data with high R^2 scores and lower error metrics. This type of combination demonstrates accuracy and precision for finding hidden relationships in morphological traits due to handling complex and nonlinear interactions (Alsharaiah et al. 2022; Park and Lek 2016). The RF model is an ensemble learning model that demonstrated better performance for chlorophyll contents and some morphological parameters, possibly due to handling noisy, multi-dimensional, and structured data (Alzraiee and Niswonger 2024; Breiman 2001). The XGBoost model is a gradient boosting framework and fitted best for predicting chlorophyll-b, indicating its superior prediction accuracy for a specific parameter. However, the performance of the XGBoost model was comparatively lower than that of the MLP and RF models (Chen and Guestrin 2016; Pathy et al. 2020). It can be concluded that ML models can be employed efficiently for predicting morphological and biochemical traits. However, the performance of these models was parameter-dependent and needed optimization through hyperparameter tuning, hybrid modeling with feature engineering, and user-friendly frameworks for improved prediction accuracy and advancements in plant biotechnology and agriculture.

Conclusion

The study investigated the impact of utilizing naturally occurring seaweed extracts as an alternative bio-stimulant for efficient and cost-effective micropropagation of *M. tweediei*. Supplementation of *J. rubens* and *C. barbata* seaweed extract to the nutrient medium resulted in enhanced micropropagation, biomass, and chlorophyll contents in *M. tweediei* without the need for expensive plant growth regulators. Moreover, the addition of 2.5 or 5% *J. rubens* extract to 2 g L^{-1} MS nutrient medium (approx. half-strength) was sufficient for a high rate of micropropagation. Eliminating the need for PGRs and using half-strength nutrient medium can reduce in vitro costs by 20–25%, resulting in significant economic gains. When *C. barbata* extract was added to the nutrient medium, the highest micropropagation results were obtained from 4 g L^{-1} MS medium (approx. full-strength) with 10% extract. However, in this medium, clump fresh weight and clump dry weight increased by 104% and 168%, respectively, compared to MS media containing *J. rubens* extract. Results were confirmed by response surface regression analysis by analyzing both seaweeds individually. Integration of ML modeling with RSM provided robust predictive insights into seaweed extracts and nutrient media composition on plant growth. Among the models tested, MLP predicted more precisely for organogenesis and biomass, while RF models predicted chlorophyll contents more precisely. Results of these findings open new avenues for sustainable and cost-effective micropropagation techniques for other aquatic plants. It is also recommended to explore the molecular mechanisms underlying the biostimulatory effects of seaweed extracts and assess their potential for large-scale commercial tissue culture systems of other plant species. Although MLP and RF models differ in their predictive strengths, both offer clear practical value for industry by enabling data-driven optimization of culture conditions and reducing reliance on costly trial-and-error experiments. Once validated, these models can be integrated into scale-up workflows to identify robust parameter ranges that maintain performance under commercial production conditions. In addition, their ability to model complex, non-linear responses allows rapid adaptation of optimized protocols to other recalcitrant aquatic species, supporting efficient process transfer and industrial implementation.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10499-026-02442-0.

Acknowledgements We thank Prof. Ayşe Nilsun Demir for her valuable advice on the study and her support in identifying seaweed species, and Burak Önol for his assistance with the chlorophyll analyses.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Esra Özcan, Seyid Amjad Ali, Muhammad Asim and Hasan Hüseyin Atar. The first draft of the manuscript was written by Esra Özcan and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK).

Data availability The data derived during artificial intelligence studies will be provided after evaluating the request.

Declarations

Ethical approval The research does not involve humans or animals, and no need for ethical approval is required.

Competing interests The authors declare no competing interests.

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