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(RESEARCH ARTICLE)

Analysis of seaweeds bio-stimulant effect on *Sedum praealtum* morphogenesis by a non-destructive distance dispersion index

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Abstract

Plant tissue culture is an important biotechnological technique for the production, conservation and regeneration of *in vitro* culture of higher plants. Biostimulants are biological, organic and synthetic components that promote plant growth and some seaweeds have been used as adequate additive for improving tissue culture as an efficient method for plant mass propagation. Modern microscopy techniques employ the management of image processing software for the extraction of numerical data from the images for better analysis. This work analyzed the biostimulant effect of marine seaweeds: *Sargassum cymosum* and *Ulva fasciata* and powders on the morphometric characteristics of *Sedum praealtum* explants by the image analysis of their shape descriptors and distance dispersion measurement validating the application of non-destructive digital morphometrics that allows describe the shape plasticity's of *S. praealtum* explants under seaweed biostimulation. This study reveals that instead of inducing a simple linear acceleration of growth; both seaweed powders function as a highly sensitive, concentration-dependent morphogenetic switch that actives the structural development and biomass quality in succulent micropropagation and mass-regeneration systems.

Keywords: Seaweeds; Biostimulants; *in vitro* Plant Culture; ImageJ; Numerical Analysis; Distance Dispersion

1. Introduction

Plant tissue culture is an important biotechnological technique due to its contribution to the production and conservation of plant-based resources [1] and the regeneration of *in vitro* culture of higher plants depending on the composition and chemical characteristics of the culture medium [2-5]. Biostimulants were considered as biological, organic and synthetic components that promote plant growth [6] and some seaweeds like *Ascophyllum nodosum*, *Fucus* spp., *Laminaria* spp., *Sargassum* spp., *Ecklonia maxima*, and *Durvillaea* spp. [7] have been used as additive for improving tissue culture of higher plants [8-13] as synthetic biofertilizers; because they contain growth regulators as cytokinins, auxins and gibberelins [14-17]. Also, the effect of various seaweed extracts and powders employed in the *in vitro* culture of plants promotes and increases the adventitious shoot regeneration on various types of explants [15,16,18]. Thus, as Ahlawat et al. [19] mention, a plant regeneration protocol with seaweed extract could be adequate for a wide range of plants cultivars and be an efficient method for mass propagation. Modern microscopy techniques generate an enormous amount of data in the form of images, and the management of image processing software allows them to extract numerical data from the images for a better analysis. ImageJ has been employed in plant biology studies to quantify and compare the cellular and subcellular characteristics [20-25]. Image processing is a powerful method in callus studies because it can distinguish intermediate levels of callus growth and therefore can provide more detailed information than conventional counting [20,26,27]. The aim of the present work was to analyze the biostimulant effect of marine seaweeds: *Ulva fasciata* and *Sargassum cymosum* powders on the morphometric characteristics of *Sedum praealtum* explants by the image analysis of their shape descriptors and distance dispersion measurement.

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2. Material and Methods

2.1. Preparation of seaweed powder

For this study, *U. fasciata* and *S. cymosum* powder were obtained from seaweeds collected in the intertidal zone at one meter of deep, in "El pulpo beach" located in Barra de Cazones, from Cazones de Herrera municipality in Veracruz, México; these were considered as 100% of seaweed dry material for the *in vitro* culture of *S. praeltum* explants.

2.2. *S. preaeltum in vitro* culture

Leaves of 3 to 5 cm from *S. praeltum* plants were cut and surface-sterilized with sodium hypochlorite solution (10%) for 10 minutes, followed by several rinses in sterile distilled water. Leaf explants were obtained aseptically cutting fractions of 0.5cm^2. Twelve explants were placed separately in standard Petri dishes (60 mm × 15 mm) containing 25mL of Murashige and Skoog (MS) medium SIGMA-M5519 [28] with the phytohormones: 1mg/L naphthalene acetic acid (NAA) + 1.5mg/L of kinetin (KIN)), supplemented with 30 g/L of sucrose and 3 g/L phytagel. The *U. fasciata* and *S. cymosum* powder were added separately at 0.25% and 0.5% to the culture medium. MS medium without any seaweed powder was used as control (0%). All the experiments were performed by duplicate and Petri dishes were sealed with Parafilm to prevent water loss and incubated at 28°C with photoperiod of 16h day/8h night cycle with a Philips Linear Fluorescent 32-Watt, 5000°K PLUS T8 Natural light bulb for 20 days.

2.3. Morphometric analysis of the seaweeds effect on *S. praelatum* explants

At the end of the tested period the *S. praealtum* explants were analyzed considering detailed images employing the Zeiss-Stemi SV11 microscope at 6X with an Axiocam HRC camera, for each experimental condition. The images were analyzed employing the ImageJ Software and the morphometric analysis was done considering the following shape descriptors: area, perimeter, centroids (x,y) and "AR" defined as the aspect ratio of the particle's fitted ellipse. Also, the distance dispersion analysis according to Uozumi et al. [21] was obtained considering the formula: $DD = (Dn\text{-}Dav)^2/A$. Where: Dn is the distance between the "x" and "y" values obtained from the centroid analysis; Dav: is the average of Dn values and A: is the area of each explant. Finally, a statistical analysis of all data was done.

2.4. Statistical analysis

All data obtained were analyzed by one-way analysis of variance and the mean differences were compared applying a Tukey-Kramer Method using the statistics program Graph Pad Instat Ver. 2.03. The "x,y" centroid relationship of each *S. praealtum* explants was also analyzed by scatter plot and the shape descriptors data: area, perimeter and AR by histogram of frequencies and matrix plot of all the numerical data by the employ of PAST software (Paleontological Statistics Software Package) Ver. 5.3.

3. Results and Discussion

3.1. Effect of *S. cymosum* and *U. fasciata* powder on *S. praeltum* explants general data

Figure 1a shows the representative culture of *S. praelatum* explants at 20 days of growth comparing the experimental conditions with the presence of *U. fasciata* and *S. cymosum* powder added to the medium and once the analysis was done. **Figures 1b, 1c and 1d** show the relative frequencies of the shape descriptors tested: area, perimeter and AR, respectively, employing a gaussian Kernel density estimation according to the rule given by Silverman [29] with missing values deleted as a smooth estimator of the histogram where selected intervals were considered. For all the experimental conditions regarding to area values there were three characteristic binds for the *S. praelatum* explants in the following order: Control 0% (8.3 to 14.12cm^2); for S-0.25% and S-0.5% (17 to 28.67cm^2); U-0.25% and U-0.5% (14.12 to 25.79cm^2). The perimeter according to the relative frequencies showed that all the experimental conditions shared the same bind values from 16.12 to 20.68cm and for the AR shape parameter with values of relative frequencies from 1.05 to 1.53cm with the same elongated form in al the *S. praelatum* explants that were grown with no drastic changes.

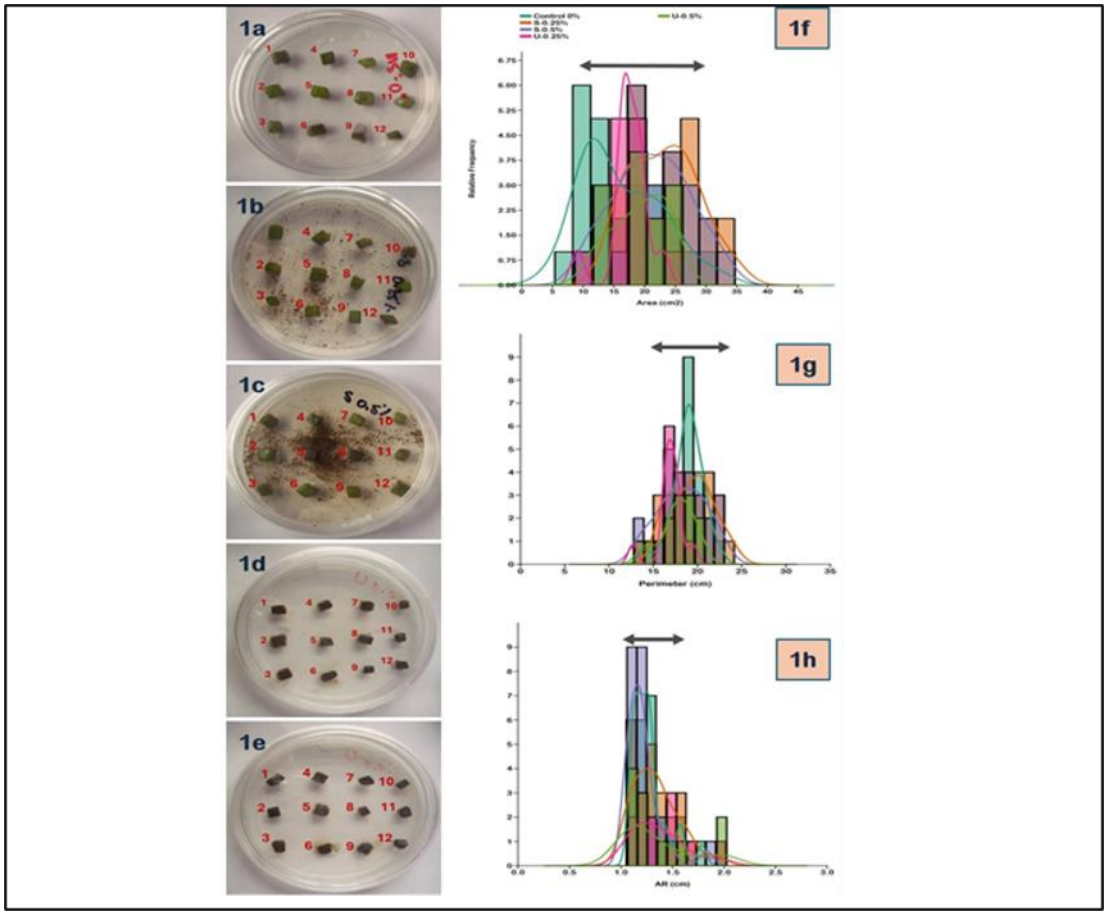

Figure 1 *In vitro S. praealtum* explants culture under: **1a)** MS-0%; **1b)** MS+S-0.25%; **1c)** MS+S-0.5%; **1d)** MS+U-0.25% and **1e)** MS+U-0.5%. Frequency histogram values: **1f)** area; **1g)** perimeter and **1h)** AR.

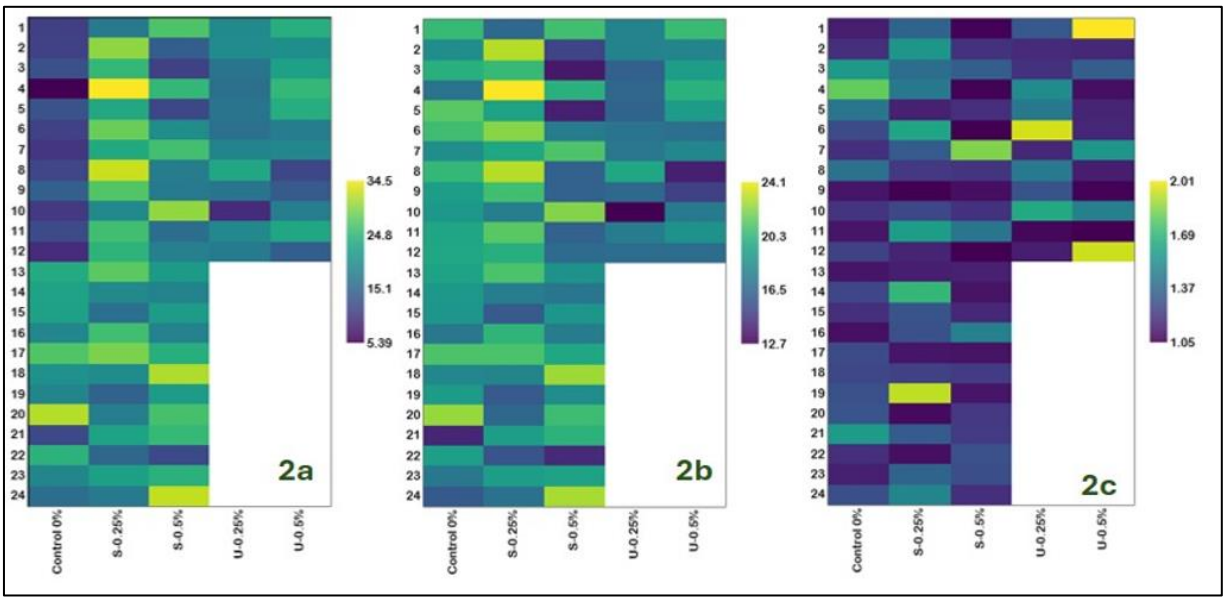

Figure 2 Matrix plots of numerical data of *S. praealtum* explants growth analysis: **2a)** area measurements; **2b)** perimeter measurements and **2c)** AR measurements.

Also, a comparative numerical data of these shape descriptors was represented in matrix plots (**Figure 2**) showing different results between explants images. Only the area values of the *S. praealtum* explants presented a statistical

significance between Control 0% (16.08±6.49cm²) with S-0.25% (23.13±5.45 cm²) ($p \leq 0.001$) and S-0.5% (21.19±5.94 cm²) ($p \leq 0.05$); S-0.25% and U-0.25% (17.40±3.25 cm²) also have statistical significance between them ($p \leq 0.05$).

3.2. Distance dispersion analysis of *S. praeltum* explants: growth extension tested

Figures 3 and 4 show the centroid relationship (x,y) of *S. praealtum* explants that allows the analysis of distance dispersion based on the distribution of centroids of *S. praealtum* explants. Under Control 0% experimental condition, the 75% of the explants were near each other with a distance dispersion value of 0.027±0.037; for the experimental condition of *S. cymosum* powder added to the medium the 83.33% and 91.66% of the explants were grouped nearest for S-0.25% and 0.5%, respectively with a distance dispersion value of 0.068±0.14 and 0.022±0.03, respectively. In presence of *U. fasciata* powder in medium, the 66.66% and 58.33% of the explants were concurrent in space with a distance dispersion value of 0.40±0.75 and 0.044±0.02 for U-0.25% and 0.5%, respectively, with a statistical significance between Control 0% and S-0.5% with U-0.25% ($p \leq 0.05$).

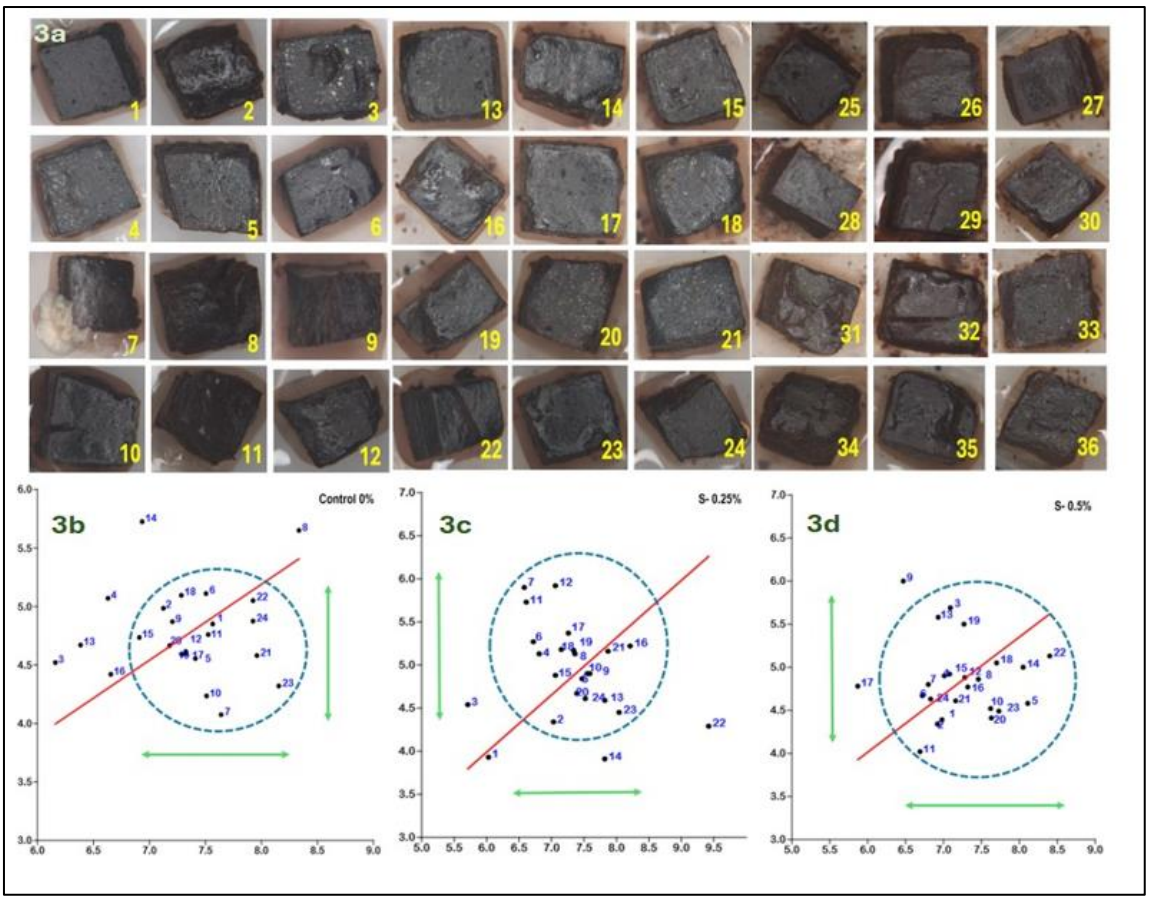

Figure 3 *S. praealtum explants growth analysis:* **3a)** explants tested with *S. cymosum* powder and scatter plot of centroid analysis: **3b)** Control 0%; **3c)** S-0.25% and **3d)** S-0.5%.

3.3. Non-destructive image analysis and effect of seaweeds on *S. praealtum* explants growth

In this work, even the *S. praealtum* explants did not showed a defined morphological change of them as a massive growth of cells dedifferentiated or disorganized or no apparent organ regeneration called as friable or compact callus as Ikeuchi et al. [30] and Frank et al. [31] described. Is important to know that this appearance could be associated with some balance between auxins and cytocinins that are involve in the determination of the differentiation and dedifferentiation estates as Iwase et al. [32] mention. Some of the explants of *S. praealtum* expanded and had white masses of incipient calli that could be considered as undifferentiated cellular "non-morphogenetic" according to Delporte et al. [33]. In this work the nature of the morphometric analysis derived from the image processing by the employ of ImageJ software, even the frequency distributions of the shape parameters analyzed: area, perimeter and AR gave a good description of probability distribution according to Mirzabie et al. [34]; there were two principal shape descriptors analyzed: the area and centroid dimensions of the explants associated by the measurement of distance dispersion data that clearly showed the effect of the presence of seaweed powder in the culture media as biostimulant. Is important to mention that the

compositions of the biostimulants varies with the types of algae (brown, green or red) and the nature of the biologically active conjugates of cytokinins and auxins could be related to the physiologically effect on *in vitro* culture of plants at low concentrations as some authors mention [16, 35-40]. The response of *S. praealtum* explants regarding to the growth of them by their dimension analysis derived from the morphometric data obtained, showed that *S. cymosum* was the biostimulant that promotes the growth of explants compared to the experimental conditions of Control and the presence of *U. fasciata* powder, showing the highest values of their area and the confidence of results by the centroid scattering plot that showed a similar response of growth under the 0.25 and 0.5% *S. cymosum* powder, also considering in the image processing and morphological analysis of the explants the measurement of the distance dispersion metric, where it allows to see how spread out were the explants regarding to its geometric center. In plant tissue culture, distance dispersion and the dispersion index are distinct computational metrics used primarily in automated computer vision and image analysis to evaluate callus growth, quality, and embryonic transition [41,42]. Particularly, regarding to explants growth the distance dispersion quantified from the centroid (x,y) and total area calculates how spread out the tissue mass is relative to its geometric center. For *S. praealtum* explants this study analyzed how they split, or scatter as a signal of their growth and the effect of the seaweed added to the medium. It is important to note that the employ of ImageJ software allows the centroid mapping, and it was useful as a standard, non-invasive morphometric tracking system. The genus *Sedum* is a succulent characterized by tightly packed, water-storing parenchyma cells. When high concentrations of hormones affect these cells, rapid, unorganized cell division (hyperplasia) occurs underneath the outer layer.

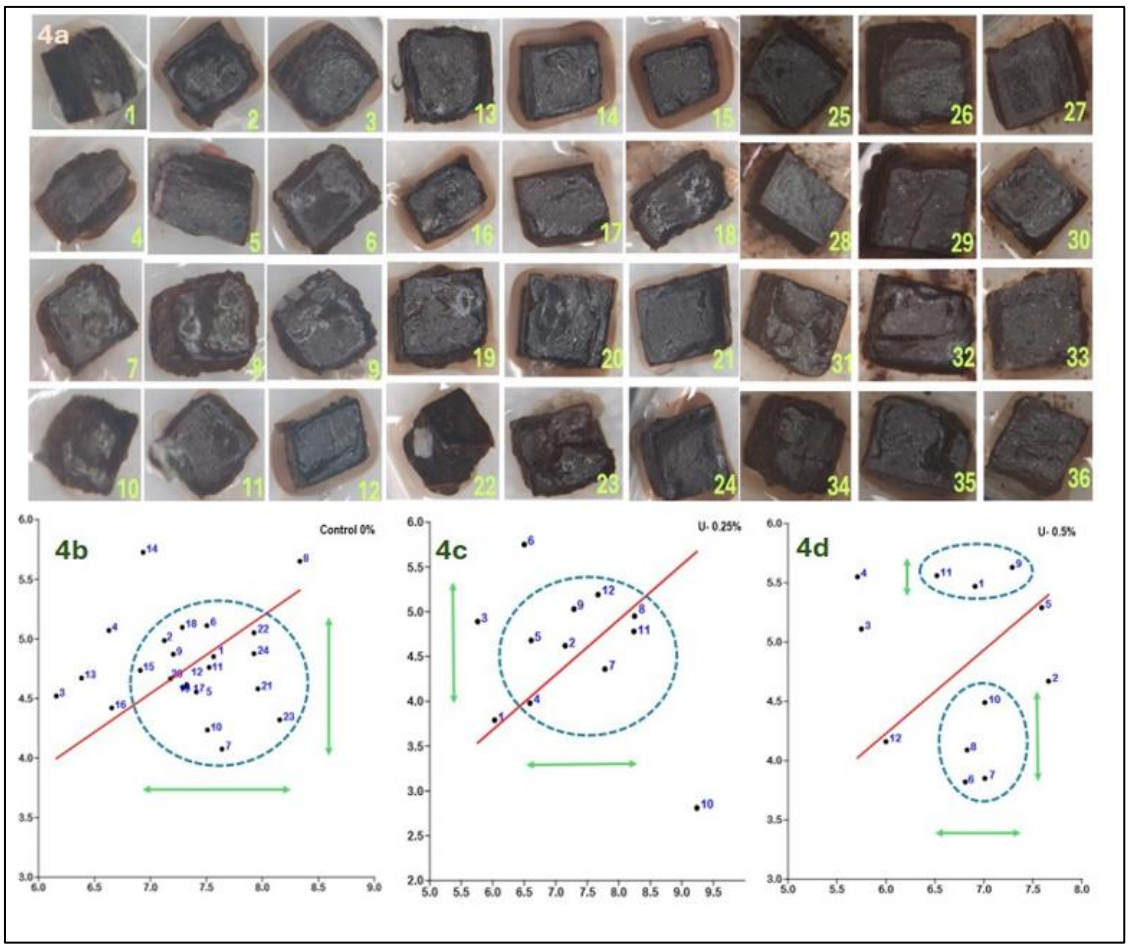

Figure 4 *S. praealtum explants growth analysis:* **4a)** explants tested with *U. fasciata* powder and scatter plot of centroid analysis: **4b)** Control 0%; **4c)** S-0.25% and **4d)** S-0.5%.

The internal physical pressure physically cracks or "splits" the explant's epidermis, causing dense clumps of callus to erupt out of localized wounds like Ikeuchi et al. [30] mention about the extensively on the genetic and physical mechanisms of plant callus induction and cell dedifferentiation. The morphometric analysis revealed a clear contrast in shape form between treatments, where the distinct morphological divergence captured by the distance dispersion index highlights the physiological impact of the seaweed biostimulants on *S. praealtum* morphogenesis. Control explants exhibited an exceptionally low normalized distance dispersion index of 0.027, reflecting an intrinsically compact,

isotropic growth pattern typical of baseline callogenesis under strict internal structural constraints. In contrast, the addition of the seaweed biostimulant powders induced a highly sensitive, biphasic, and concentration-dependent structural modulation. At a lower inclusion rate of 0.25%, both macroalgae broke this baseline rigidity, causing the dispersion index to rise to 0.068 in *S. cymosum* and to a notable peak of 0.400 in *U. fasciata*. This initial shift quantitatively demonstrates a transition toward expansive peripheral growth and structural relaxation, implying that lower quantity of biostimulant components promotes loose outward cell elongation. However, doubling the concentration to 0.50% activated a strict physiological threshold that triggered a profound structural re-compaction. Under this higher dosage, the distance dispersion indices collapsed drastically to 0.022 in *S. cymosum* and 0.044 in *U. fasciata*, effectively returning the tissues to a hyper-dense state close to or exceeding the control compaction level. Furthermore, because the biostimulant was applied as a solid powder matrix within the medium, these ultra-low dispersion values under high doses suggest that the explants may be responding to localized micro-environmental gradients or physical anchoring sites created by the powder particles, forcing highly concentrated, aggregate cellular proliferation in localized sites of the explants.

3.4. Synergy between biomass accumulation and structural compaction

A highly remarkable finding in this study is the inverse relationship observed between tissue area and the distance dispersion index under the higher seaweed biostimulant treatments (0.50%). While the control explants maintained a baseline size with an intrinsically low and compact dispersion of 0.027, the 0.50% biostimulant-treated *S. praealtum* explants simultaneously achieved the highest total surface area and the lowest distance dispersion indices (0.022 for *S. cymosum* and 0.044 for *U. fasciata*). In standard plant morphometrics, an increase in tissue area typically correlates with an increased or highly irregular spatial dispersion due to peripheral cell stretching or asymmetrical branching. However, the combination of maximum area and near-zero dispersion mathematically demonstrates a phenomenon of hyper-dense biomass packing. This implies that high concentrations of the seaweed biostimulant powder do not merely trigger unorganized cell elongation; rather, they stimulate a massive upregulation in localized cell division. Consequently, the high-concentration powder matrix creates a highly efficient micro-environment where the Sedum explant can maximize its biosynthetic mass (high area) while remaining structurally fortified and tightly organized (low dispersion).

3.5. Comparative morphological modulation by *S. cymosum* and *U. fasciata* biostimulants

The morphometric shape profiling executed via ImageJ revealed distinct, genus-specific, and dose-dependent developmental pathways in *S. praealtum* explants. Both *S. cymosum* and *U. fasciata* elicited a classic biphasic or threshold-dependent morphogenetic response, using the control group (0.027) as a highly compact structural baseline. At the lower inclusion rate of 0.25% powder, both seaweeds triggered structural relaxation, driving the distance dispersion index upward to 0.068 (*S. cymosum*) and a prominent 0.400 (*U. fasciata*). These values, being above the control baseline, demonstrate a loose, asymmetrical expansion or peripheral cellular stretching, a phenotypical plasticity likely promoted by the specific auxin-to-cytokinin ratios and mineral profiles inherent to low-dose macroalgae application. However, doubling the biostimulant concentration to 0.50% induced a drastic morphological pivot across both genera, causing the dispersion indices to collapse to 0.022 and 0.044, respectively. This sudden shift indicates that a physiological level was crossed, wherein high concentrations of seaweed bioactives (such as complex alginates and fucoidans in *S. cymosum*, and sulfated ulvans in *U. fasciata*) suppress loose elongation and trigger sudden structural re-compaction. Collectively, these digital morphometric profiles prove that both seaweeds function as tunable morphogenetic switches-capable of promoting loose spatial expansion and cellular stretching at lower doses, and hyper-compact tissue aggregation paired with massive biomass accumulation at higher concentrations.

3.6. Concentration-dependent morphological regulation by seaweed powders

The digital morphometric analysis revealed that both *S. cymosum* and *U. fasciata* biostimulants regulate the shape form of *S. preaeltum* explants via concentration-dependent pathways, though they follow distinct developmental trajectories based on their baseline divergence. The control group established a compact baseline index of 0.027, representing strict, localized radial expansion. The addition of seaweed powders at 0.25% broke this constraint, inducing a relaxation of tissue architecture that allowed greater spatial dispersion. In clear contrast to this low-dose expansion, increasing the dosage to 0.50% triggered a strict stepwise structural re-compaction. Coupled with the fact that these 0.50% treatments yielded the highest total explant area, this dramatic reduction mathematically proves a direct dose-dependent threshold: higher concentrations of marine biostimulants pack an increasingly massive volume of cells into tighter, structures without allowing peripheral cell elongation. This divergence demonstrates that marine biostimulants function as concentration-sensitive developmental switches-promoting expansive cellular stretching at lower thresholds before triggering severe structural compaction and hyper-dense biomass allocation at higher concentrations.

4. Conclusions

This study successfully validated the application of non-destructive digital morphometrics, and the distance dispersion index extracted via ImageJ-to map the intricate shape plasticity's of *Sedum praealtum* explants under seaweed biostimulation. Our datasets reveal that instead of inducing a simple linear acceleration of growth; both *Sargassum cymosum* and *Ulva fasciata* powders function as a highly sensitive, concentration-dependent morphogenetic switch. While the control explants exhibit an intrinsically compact baseline (0.027), a low concentration (0.25%) of seaweed powder successfully breaks this structural constraint to promote expansive, peripheral cell stretching and relaxed tissue architecture, reaching a prominent dispersion peak of 0.400 with *U. fasciata*. Conversely, a higher concentration (0.50%) activates a strict physiological response that collapses spatial dispersion (down to 0.022 in *S. cymosum* and 0.044 in *U. fasciata*) while simultaneously maximizing total tissue surface area. This state mathematically confirms the induction of tightly cohesive cells driven by localized hyperplasia rather than unorganized loose proliferation. Ultimately, integrating automated shape profiling into tissue culture protocols allows researchers to dynamically track and predict explant quality without destroying the live tissue. This work establishes a clear, quantifiable baseline for optimizing the dosage of macroalgae biostimulants to active the structural development and biomass quality in succulent micropropagation and mass-regeneration systems.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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