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Effect of Ulva fasciata powder as biostimulant on Sedum praealtum *in vitro* growth

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Abstract

Biostimulants are considered as biological, organic and synthetic components that promote the plant growth and also they have been used as an additive for improving in the plant tissue culture technology the successful initiation, regeneration and micropropagation of *in vitro* culture of higher plants The aim of the present work was to analyze the effect of *Ulva fasciata* seaweed powder on the growth and differentiation of *Sedum praealtum in vitro* culture. The measurements of the area and perimeter descriptors showed a particularly positive effect at 0.25% *U. fasciata* powder, followed by the experimental condition of culture with 0.5% *U. fasciata* powder with more area and perimeter than control explants, respectively with statistical significance. Regarding to the morphometric relationship between aspect ratio (AR) and roundness of the explants, all of the experimental conditions with and without the *U. fasciata* powder indicated that all of *S. praealtum* explants were closer to the ellipsoidal values than to the roundness. Also in this study, not only the nature of phytohormones present in the media tested (naphthalene acetic acid and kinetin) for *S. praealtum* explants culture were determinant; also the addition of seaweed powder may be promoted the growth of them and also an initial changes of morphogenesis with the evidence of an incipient callus.

Keywords: Seaweeds; Biostimulants; *In Vitro* Plant Culture; *Sedum praealtum*; *Ulva fasciata*

1 Introduction

Biostimulants were considered as biological, organic and synthetic components that promote the plant growth [1]; particularly, seaweed extracts and powders from a wide range of seaweeds such as *Ascophyllum nodosum*, *Fucus* spp., *Laminaria* spp., *Sargassum* spp., *Ecklonia maxima*, and *Durvillaea* spp. have been employed as substitute of synthetic fertilizers; because they stimulate plant growth [1-13] and their chemical analyses revealed the presence of a wide variety of plant growth promoting regulators [14] as gibberellins [15-17], auxins [18,19] and cytokinins [20,21]. In the plant tissue culture technology, the successful initiation, regeneration and micropropagation of *in vitro* culture of higher plants depend on the composition and chemical characteristics of the culture medium. Thus, seaweeds have been used as additive for improving tissue culture of higher plants [8, 22- 26]. The aim of the present work was to analyze the effect of *Ulva fasciata* seaweed powder on the growth and differentiation of *Sedum praealtum in vitro* culture.

2 Material and Methods

2.1. Preparation of seaweed powder

For this study, *Ulva fasciata* specimens were hand collected from 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; cleaned several times with sea water to remove sand, other impurities and epiphytes, then transported to laboratory and again cleaned four

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times with tap water and finally shade dried. This shade-dried seaweed was finely chopped and powdered with a Nutribullet®. Finally, the powder was considered as 100% of seaweed dry material and stored at 4°C for the *in vitro* culture of *Sedum praealtum* explants.

2.2. *Sedum praealtum in vitro* culture

Leaves of 3 to 5 cm from *Sedum praealtum* 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 [27] with the phytohormones: 1mg/L naphthalene acetic acid (NAA) + 1.5 mg/L of kinetin (KIN)), supplemented with 30 g/L of sucrose and 3 g/L phytagel. The *U. fasciata* powder tested was added at different concentrations 0 (Control), 0.25% and 0.5% to the culture medium. 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. Analysis of the *U. fasciata* powder effect on *Sedum praealtum* explants

At first, Petri dishes containing the *Sedum praealtum* explants were photographed using Kodak Easyshare C713 Zoom Digital Camera (3X, 7.0 MPixels at 20cm) and each explants from the experimental images were analyzed employing the ImageJ Software and the morphometric data (area, perimeter, circularity (with a value of 1 indicating a perfect circle. As the value approaches 0, it indicates an increasingly elongated shape), Feret diameter (The longest distance between any two points along the selection boundary, also known as maximum caliper), aspect ratio ("AR" is the aspect ratio of the particle's fitted ellipse) and roundness (the inverse of AR)) were obtained and exported as excel files and finally a statistical analysis of individual explants was done. Also, explants detailed images were obtained employing the Zeiss-Stemi SV11 microscope at 6X with an Axiocam HRC camera, for their analysis regarding to their development and growth in each experimental condition.

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 relationship between the roundness and AR of *Sedum praealtum* explants was also analyzed by regression analysis and a Principal Component Analysis (PCA) was done considering the shape descriptors: area, perimeter, circularity, Feret diameter, AR and roundness; measuring its variance-covariance correlation both analyses done by the employ of PAST software (Paleontological Statistics Software Package) Ver. 4.09.

3 Results and Discussion

3.1. Effect of *Ulva fasciata* powder on *Sedum praealtum* explants morphometric analysis

Figure 1 shows the *Sedum praealtum* explants measured at 20 days of growth comparing the experimental conditions with the presence of *U. fasciata* powder added to the medium.

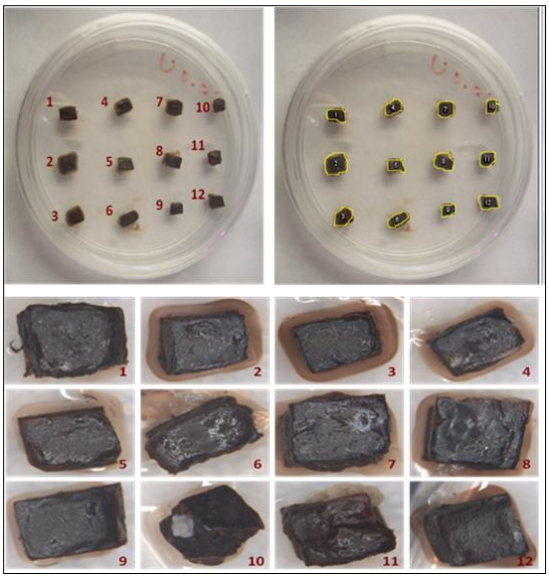

Figure 1 *In vitro Sedum praealtum* explants culture under MS+U0.25% medium and explants detailed growth and appearance

Figure 2 shows the particularly measurements comparing the area and perimeter descriptors; showing a particularly positive effect at 0.25% *U. fasciata* powder (263% and 202% more area and perimeter than control explants, respectively); followed by the experimental condition of culture with 0.5% *U. fasciata* powder (152% and 135% more area and perimeter than control explants, respectively) with statistical significance ($p<0.001$).

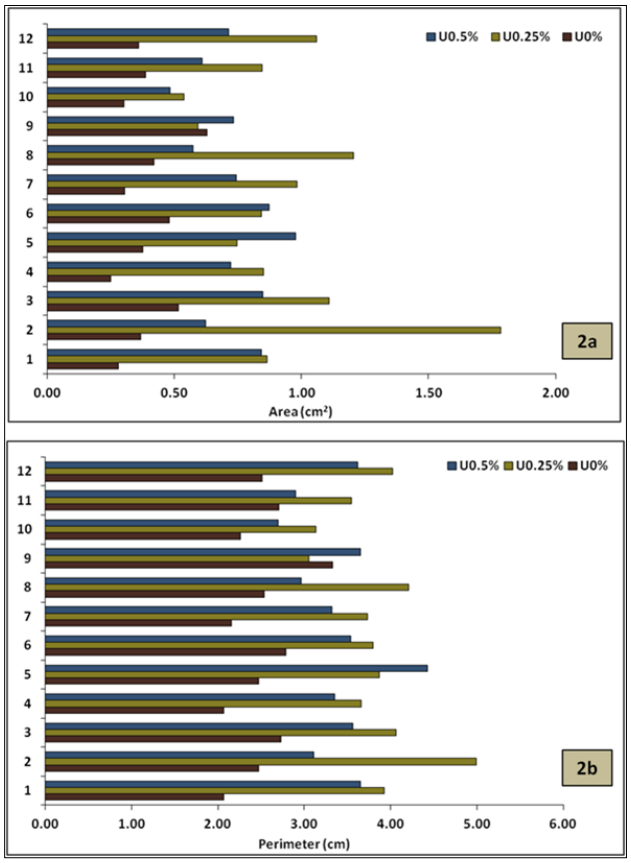

Figure 2 *Sedum praealtum explants growth analysis:* **2a)** area values and **2b)** perimeter values (n = 12 explants)

Regarding to the morphometric relationship between AR and roundness of the explants, Figure 3 shows the regression analysis of the experimental conditions with and without the *U. fasciata* powder; all figures indicated that explants are closer to the AR values that roundness, conserving these values in all the experimental conditions and growth of *Sedum praealtum* explants (U0%: r = 0.99; U0.25%: r = 0.92; U0.5%: r =0.98).

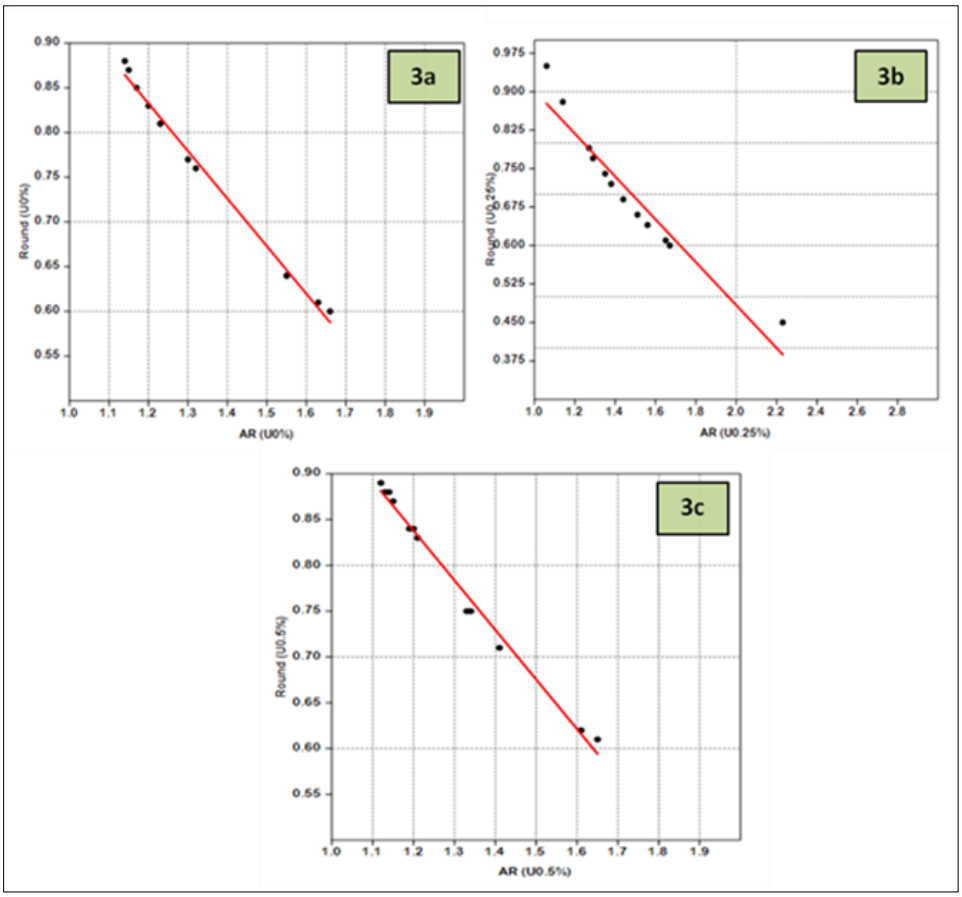

Figure 3 Linear regression curves showing the relationship between *Sedum praealtum* explants roundness and aspect ratio (AR): **3a)** U0% (r = 0.99), **3b)** U0.25% (r =0.92); **3c)** U0.5% (r = 0.98). Groups of individual explants are shown in the plots

3.2. Relationship between experimental conditions and *Sedum praealtum* explants response

Finally, according to the multivariate analysis as a complement to the study, Figure 4 showed a separation of the experimental conditions tested according to the medium culture; where Component 1 values (98.15%) indicates a separated analysis of U0% condition related with the morphometric parameters: AR, roundness and circularity and in this figure a particularly association between the experimental conditions: U0.25% and U0.5% with area and Feret diameter (Component 2 values 1.76%). In this study, is important to note that according to Lakshmanan et al. [28] and Smolenskaya et al. [29] whose reported that the presence of particularly growth regulators and their concentrations are important factors and the effect of growth regulator on tissue cultures can vary according to the chemical nature of the compound, plant species, type of culture and even the developmental state of the explant. In this study, not only the nature of phytohormones assayed in *Sedum praealtum* explants in the media tested (NAA and KIN) were determinant; also the addition of seaweed powder may be promoted the growth of them and also an initial changes of morphogenesis.

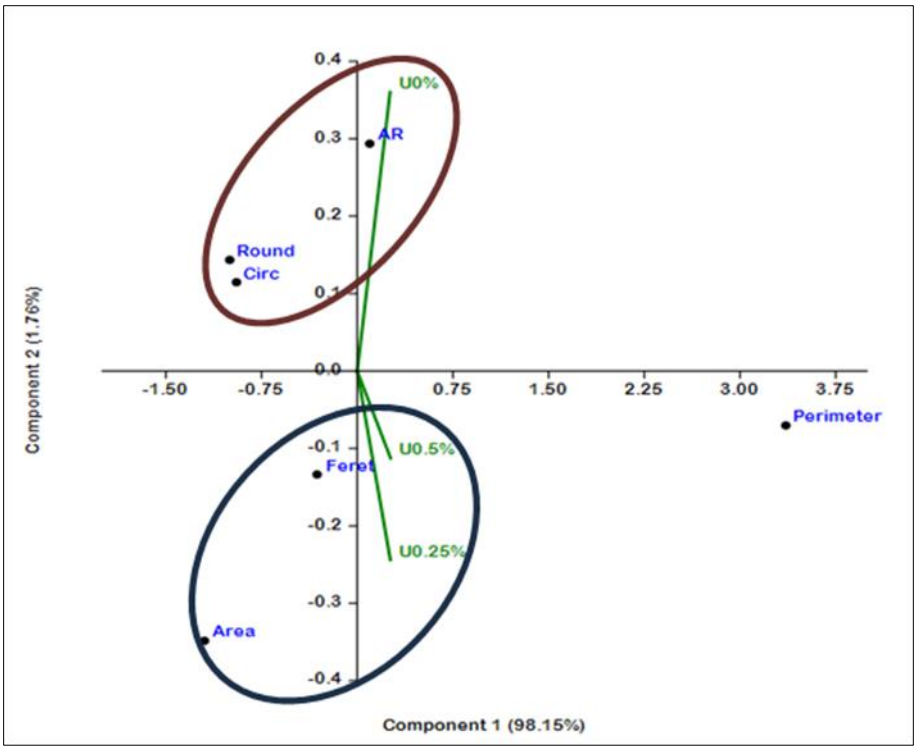

Figure 4 Principal component analysis grouping the *Sedum praealtum* the morphometric parameters and experimental conditions

3.3. Morphological changes induced in *Sedum praealtum* explants

Ikeuchi et al. [30] defined "callus" to the massive growth of cells and also applied to the disorganized plant cell masses (calli) and also established that calli are diverse and could be particularly classified based on their macroscopic characteristics. Authors like Zimmerman [31] and Frank et al. [32] described two principal kinds of calli; one with no apparent organ regeneration called as friable or compact callus and the other that shows some degrees of organ regeneration called rooty, shooty, or embryonic callus. Iwase et al. [33] also mention that this term must include cells with various degrees of differentiation when the balance between auxins and cytokinins determines the state of differentiation and dedifferentiation. In this work Figure 1 shows the appearance of the *Sedum praealtum* explants analyzed under the experimental condition of U0.25% where some of them are expanded and it is clear the presence of white masses that could be considered as an initial callus. According to Delporte et al. [34] these authors established that "callogenesis" is characterized by undifferentiated cellular proliferation, considering calli as "non-morphogenetic" with white, limpid, watery and friable appearance.

4 Conclusion

In this work, the presence of seaweed powder in a culture medium showed that it could be considered a particular additive and maybe could be considered as a plant growth regulator showing an effect on the growth of *Sedum praealtum* explants and also be involved in the initial differentiation response to the callus formation in the explants tested.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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