Image Analysis of Root Induction in *Sedum praealtum* Cuttings

Ana K. Hernández-Zamora, Angélica Rodríguez-Dorantes

**ABSTRACT**

Production of adventive roots is a process induced and regulated by phytohormones where auxins play an important role in controlling the growth and development of them, with a direct influence on the regenerative ability of the plant. The aim of this study was to compare the root induction and development on *Sedum praealtum* cuttings by the action of 3-indolylbutyric acid (IBA) and naphthalacetic acid (NAA) concentrations by root image analysis. Root length of plantlets showed that there was an interval between 0.1 and 1.0 mg/L concentrations of efficient root induction with a clearly decrease of it as IBA concentration increased, also as NAA treatment showed. In this work, the rooting response of *Sedum praealtum* cuttings evaluated by root image analysis showed the application of IBA as an efficient synthetic auxin for vegetative propagation.

**Keywords:** *Sedum praealtum*, cuttings, plant growth regulators, roots.

I. INTRODUCTION

Production of adventive roots is a process induced and regulated by plant growth regulators and it has a direct influence on the regenerative ability of the plant. Auxins play an important role in controlling the growth and development of the plants; particularly, in primary, secondary and adventitious roots production [1] and they are currently employed for rooting of cuttings. Synthetic 3-indolylbutyric acid (IBA) has been the most commonly used auxin, because it promotes the production of adventitious roots compared to natural indolyl-3-acetic acid (IAA). Another relatively common synthetic auxin employed is α-naphthylacetic acid (NAA); even it is not so effective on plant rooting, it possesses the advantage of lower price [2].

The genus *Sedum* (Crassulaceae) comprises almost 400 species, distributes all over the world except Australia and the Pacific islands and some of them are known as CAM (Crassulacean Acid Metabolism) plants [3]-[5]. There has been an important report about *Sedum roseum* and *Sedum drymarioides* regeneration [6], [7], and also a study about the efficient micropropagation system of *Sedum alfredii*, as a plant hyperaccumulator of zinc and cadmium [8]-[10].

Considering the importance to the establishment of vegetative propagation of some species of this genus, the aim of this study was to compare the effect of IBA and NAA plant growth regulators on *Sedum praealtum* roots induction, by root image analysis.

II. MATERIALS AND METHODS

A. Plant Root Induction of *Sedum praealtum*

Among 25 selected *Sedum praealtum* plants were collected from the gardens of E.N.C.B., and 5cm of length cuttings were obtained from these plants containing buds and 3 leaves. These were surface sterilized at stem cut zone with sodium hypochlorite (10%) for 5 minutes, rinsed with sterile distilled water and placed separately in sterile baby food flasks with Magenta SIGMA caps containing 90mL of concentrate mineral medium (0.20 M NH₄H₂PO₄, 1.15 M Ca(NO₃)₂, 0.26 M CaCl₂, 0.40 M MgSO₄·7H₂O, 1.2 M KNO₃, 1.2 × 10⁻³ M H₂BO₃, 1.2 × 10⁻¹ M CuCl₂·H₂O, 2.3 × 10⁻³ M ZnCl₂, 4.4 × 10⁻⁴ M MnCl₂·4H₂O, 6 × 10⁻⁴ M Na₂MoO₄·H₂O, 7.2 × 10⁻⁵ M EDTA·Na₂ and 7.1 × 10⁻⁴M FeSO₄·7H₂O·pH=7.6). Control plants cuttings were considered without plant growth regulators added and the root induction was considered adding IBA at 0.1, 0.5, 1.0, 1.5, 2.0 and 3.0 mg/L concentrations, also NAA was tested only at 1mg/L concentration. All of these hydroponic cultures were cover with paper favoring the root induction and growth, performed by triplicate and maintained under greenhouse conditions for 30 days. At the end of this time, plants were obtained, and each was analyzed regarding to their development in each experimental condition. At first, roots images were obtained using Kodak Easyshare C713 Zoom Digital Camera and these images were analyzed employing the Motic Image 2000 Software Ver. 1.3, standardizing their size and after all the roots developed were lineally measured and the summary of all were obtained.

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B. Statistical Analysis

All data 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 root length and roots number obtained in each experimental condition was also analyzed by regression analysis using the Paleontological Statistics Software PAST Ver. 2.17b.

III. RESULTS

A. Root induction in Sedum prealtum cuttings

Fig. 1 shows the experimental conditions tested for S. prealtum cuttings; these were organized inside a chamber under greenhouse conditions (Fig. 1a) and flasks were covered to avoid light interferences on root induction. Fig. 1b shows how root images were processed and analyzed by Motic Image Software.

The effect of IBA on S. prealtum induction showed that the number of roots increased in 0.1, 1.0 and 2mg/L concentrations compared to control plant cuttings, where the number of roots diminished as NAA experiments (Fig. 2).

Root length of S. prealtum plantlets showed that there was an interval between 0.1 and 1.0 mg/L concentrations of efficient root induction; clearly the increase in IBA concentration decrease the length of roots as control and NAA did, with a statistical significance between them (Fig. 3a, p< 0.001). Fig. 3b shows the correlation between root length and roots number obtained in each experimental condition; with an evident behavior of two middle IBA concentrations tested (0.1 and 1.0 mg/L) and the diminished effect of the increased IBA concentrations (1.5, 2.0 and 3.0 mg/L).

It is important to note that even the high number of roots produced, these were shortly and thick with necropsied apical meristems as the appearance obtained with NAA; where this plant growth regulator slightly diminished the number of roots produced in S. prealtum cuttings. The particular response of S. prealtum root length profiles in Fig. 4, reveals that this parameter was more continuous between cuttings tested in 0.1 mg/L; compared to cuttings in 1.0 mg/L of IBA plant growth regulator, with a better growth of plants in the lowest concentration.
The effects of auxin group of phytohormones on rooting and plant development have been discussed in several studies: Stefančič et al. [11] studied the effectiveness of IBA and NAA in Pseudotsuga menziesii; Hossain et al. [12] analyzed the effectiveness of IBA in Swietenia macrophylla and Chukrasia velutina; Chhun et al. [13] researched the effectiveness of IAA, IBA, and NAA in Oryza sativa; de Klerk et al. [14] analyzed the effectiveness of IAA, IBA, and NAA in Malus sp.; Martin [15] studied the effectiveness of IBA in Holostemma addkodien; Tchoundjeu et al. [16] analyzed the effectiveness of IBA in Prunus Africana; Swamy et al. [17] studied the effectiveness of IBA and NAA in both Robinia pseudoacacia and Grewia optiva. All of these studies show in general auxin group of hormones has an open and wide effect on plants rooting initiation. Thus, adventitious root formation is influenced by internal and external factors were natural and synthetic auxins regulated this process [18]. Particularly, Zhao et al. [19] analyzed the efficient regeneration system of Sedum alfredii using stem and leaf tissues; they reported that this three plant growth regulators: IBA, IAA, and NAA, induced rooting of elongated shoots within a period of 2 weeks. However, differences in efficiency of rooting induction were observed among them: IBA induced the highest frequency of rooting (100%) at all tested concentrations (0.1, 0.5, 1.0, 1.5, 2.0 and 3.0 mg/L), followed by IAA and then NAA, authors also noted roots induced by NAA, at 0.1, 0.5, 1.0, 2.0 and 3.0 mg/L concentrations, were rather short, thick and plantlets were grown slowly. In this work, S. praealtum plantlets showed a similar response compared to S. alfredii regarding to the efficiency of IBA, but only at lowest concentrations tested.

### IV. DISCUSSION

Vegetative propagation of some species ensures the preservation of all characteristics of the parent plant and depends on the regeneration ability and growing of adventitious roots in plant cuttings. In this work, the rooting response of Sedum praealtum cuttings evaluated by root image analysis showed the application of indolybutyric acid was an efficient synthetic auxin for its vegetative propagation.

### V. CONCLUSION

Vegetative propagation of some species ensures the preservation of all characteristics of the parent plant and depends on the regeneration ability and growing of adventitious roots in plant cuttings. In this work, the rooting response of Sedum praealtum cuttings evaluated by root image analysis showed the application of indolybutyric acid was an efficient synthetic auxin for its vegetative propagation.

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