



Article

Enhancing Propagation of *Orostachys* spp. Offsets through Exogenous GA₃ Application

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Abstract: *Orostachys* spp., considered economically important succulent plants for both the medicinal and ornamental plant industries, are known to exhibit slow growth in their offsets during the long, cold winter months. Due to the slow growth, this study investigated the application of gibberellic acid (GA₃) and determined the optimal GA₃ concentration (control, 200 mg·L⁻¹, 400 mg·L⁻¹, and 600 mg·L⁻¹) for promoting the propagation of three *Orostachys* species (i.e., *O. fimbriata*, *O. japonica*, and *O. minuta*), as well as its impact on their growth and development. According to our study findings, *O. fimbriata* and *O. minuta* influenced by GA₃ exhibited higher survival rates (7–38%) and offset growth rates (3–87%) compared to the control. Similarly, its application resulted in significant shoot and root development, along with increased moisture content for the majority of the species. The results of this research demonstrate the potential and practical applications of using GA₃ to increase the propagation and growth of *Orostachys* spp. during cold conditions for year-round propagation of these succulent plants, which could have ecological and horticultural significance for related species as well as other vegetatively propagated crops.

Keywords: cultivation; GA₃; medicinal plants; offset; succulents



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1. Introduction

A member of the Crassulaceae family, the genus *Orostachys* is composed of 12 recognized perennial succulent species that are abundantly grown in Korea, Japan, and Russia and operate via Crassulacean acid metabolism (CAM), which allows them to easily adapt to drought, cold, and stressful environments [1,2].

Several species of the *Orostachys* genus are economically important as high-valued ornamental potted plants (*O. ‘Nungyu bawisol’*) [3], ground covers (*O. japonica*) [4], landscape flower beds (*O. japonica*, *O. minuta*, and *O. chongsunensis*) [5], and green roofing plants (*O. fimbriata*) due to their small and exquisite leaves and formation of beautiful offsets [6]. Aside from its horticultural purpose, this genus has recently risen in popularity as a source of food and medicine [2]. Thus, many studies have engaged in characterizing and developing purification methods of *Orostachys* species as a rich source of flavonoids (i.e., epicatechin gallate, quercetin, and kaempferol) [7,8], anti-inflammatory novel polysaccharide, anti-tumor, antioxidant, and antifebrile components (i.e., 4- α -GlcP-(1 and 4,6)- β -GlcP-(1)) [9–11].

Because of their medicinal benefits, there is a large demand, especially in China, for *Orostachys* products and raw materials for their production, which are derived from different wild species [12]. However, the wild resources of these species are scarce and have already been endangered; hence, there is a need for improved propagation techniques to meet its demand as well as maintain progenies for conservation [13].

Succulents, such as *Orostachys* species, are commonly propagated through axillary buds attached to the leaves, stems, and offsets. These buds are attached to the stems, grow outward, detach, and should grow separately, but they are highly dormant [14].

They have a natural dormancy period wherein these species slow down their growth and are triggered by environmental factors that usually occur during winter and thus also affect plant propagules for propagation [14–16]. In order to establish a large-scale and less sophisticated solution to this barrier in propagation, the use of exogenous hormones is commonly applied to the propagules prior to planting [17].

Among the key plant growth regulators, gibberellins (GAs) have emerged as pivotal hormones with remarkable effects on plant propagation, as they are integral in the development of seeds and fruits and the primary hormone controlling plant height, shoot, and root elongation [18]. In recent years, the application of exogenous gibberellins has garnered significant attention as a promising strategy to modulate plant physiology such as those with problems in lagged growth and triggering branching. The application of GA is more prominently used to increase growth rates in various crops in in vivo and in vitro conditions [19–21]. The main mechanism by which GA expedites growth is by targeting the intercalary meristem as well as affecting gene transcription for specific steps in the biosynthetic pathways to mediate environmental factors such as temperature and photoperiod [22].

Studies have also reported that the exogenous application of GA has also encouraged growth in vegetative propagules of several economically important crops such as *Allium sativum* cloves [23], *Rhododendron* species flower buds [24], detached apple shoots [25], and strawberry petioles [26], among others. Lang et al. [27] explained that lagged or slower growth rates are affected by seasonal changes that are indicative of temperature extremes during winter and chilling and/or photoperiodic responses during autumn. As a complex process necessary for plant survival, propagation, and development, dormancy regulation in vegetative buds is highly associated with the action of specific hormones [28]. In plant models for vegetative propagation, among other plant hormones, GA is known to promote growth through the upregulation of D-type cyclins and cyclin-dependent kinase as well as A- and B-type cyclins [29], which are involved in G1-S and G2-M and are paramount to the growth and progression of bud spurge overcoming inhibiting environmental factors experienced by plant propagules being propagated in colder months [30,31].

However, the majority of the studies on *Orostachys* species propagation with the application of exogenous plant growth regulators have used in vitro methods such as tissue culture [13,32,33]. Several plant nurseries do not have sophisticated facilities to propagate using controlled sterile conditions grown in artificial media, and using exogenous hormones is the most suitable way to improve propagation efficiency.

Hence, in this study, the use of the exogenous application of GA₃ at different concentrations on the offsets of three economically important *Orostachys* species is investigated to determine its effect on their propagation, growth, and development.

2. Materials and Methods

2.1. Plant Materials

Three *Orostachys* species (Figure 1), namely, *O. fimbriata*, *O. japonica*, and *O. minima*, were utilized in this study and were brought to the Experiment Greenhouse of the Department of Environmental Horticulture, Sahmyook University, South Korea.

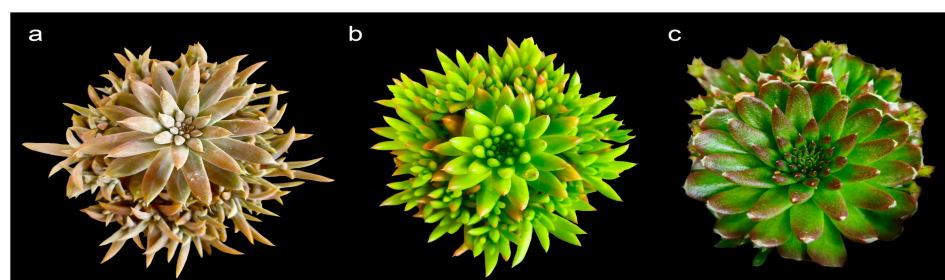


Figure 1. *Orostachys* species used in this study: (a) *O. fimbriata*; (b) *O. japonica*; and (c) *O. minima*.

2.2. Experimental Design and Treatment Applications

To determine the optimized concentration for breaking the dormancy of *Orostachys* propagules, we used gibberellic acid (GA₃; CAS No. 77-06-5, Sigma Aldrich, St. Louis, USA) at different concentrations. The following concentrations were used as treatments: control (no exogenous GA₃ application), 200 mg·L⁻¹, 400 mg·L⁻¹, and 600 mg·L⁻¹. Due to the limited supply of these wild species, each treatment was replicated three times with 12 plants per replication treatment.

Mother plants with 1 cm-sized offsets were detached (Figure 2) and collected during the autumn season, which is considered the onset of lagged growth for the plant propagules. The offsets were planted in trays (28.0 × 52.0 × 4.5 cm; L × W × H) having 72 individual cells (45 cc per cell) that were filled with horticultural substrates (Hanareumsagto, Shinsung Mineral, Goesan-gun, Republic of Korea) as a potting medium. Exogenous application of GA₃ was carried out using the drenching method with 10 mL solution of their respective treatment concentrations at planting.

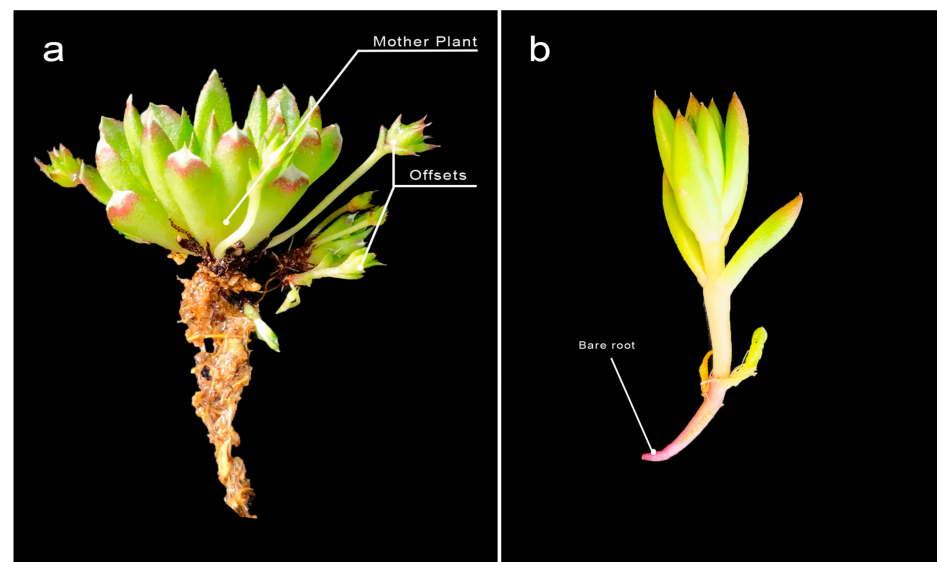


Figure 2. Plant propagules of an *Orostachys* species showing: (a) the mother plant with several attached offsets growing at its basal area (*O. minuta*); and (b) the detached offset as bare root, which was used as propagule (*O. japonica*).

2.3. Data Gathered

The survival rate was calculated by counting the number of successfully developed offsets over the initial number of planted propagules and is presented as percentages, while the offset growth rate was measured by calculating the percentage increase over time. Other plant growth and development parameters were also gathered, such as shoot length and width, stem diameter, root length, number of offsets, offset length, leaf length and width, and ground cover.

Fresh weight was measured by removing the soil attached to the plant, rinsing the plant with water, and then allowing it to air-dry naturally in a sealed space for 24 h before measurement. Dry weight was measured after hot-air drying the samples in a dry oven (HK-DO135F, Hankook S&I, Republic of Korea) at 85 °C for 24 h. To determine the moisture content (MC), it was finally calculated using this formula: % MC = [(A−B)/A] × 100, where A is the fresh weight and B is the dry weight.

2.4. Care and Management

The offsets were placed inside the greenhouse with controlled conditions with a recorded temperature of 20.4 °C ± 2.1 and relative humidity of 45.8% ± 13.8 throughout

the duration of the study. Overhead watering was carried out three times a week for water management.

2.5. Statistical Analysis

Numerical data obtained from *Orostachys* species were subjected to analysis of variance (ANOVA). Significant differences between means were analyzed using Duncan's multiple range test at a 5% significance. All statistical analyses were performed using SAS 9.4 (SAS Institute, USA).

3. Results

3.1. Survival Rate and Growth

The exogenous application of gibberellic acid (GA_3) had a significant impact on the survival rate (Figure 3) and offset growth rate (Figure 4) of *Orostachys* species. In *O. fimbriata*, control plants had the lowest survival rate of 66%, while propagules treated with GA_3 had significantly higher survival rates with the use of $400\text{ mg}\cdot\text{L}^{-1}$ (88%), $600\text{ mg}\cdot\text{L}^{-1}$ (83%), and $200\text{ mg}\cdot\text{L}^{-1}$ (72%) (Figure 3A). Among the species, only *O. japonica* showed no significant differences after the application of GA_3 , which had a 100% survival rate three months after treatment (MAT) (Figure 3B). On the other hand, *O. minuta* treated with GA_3 , regardless of the concentration, had the highest survival rates (94%) compared to the control (66%) (Figure 3C).

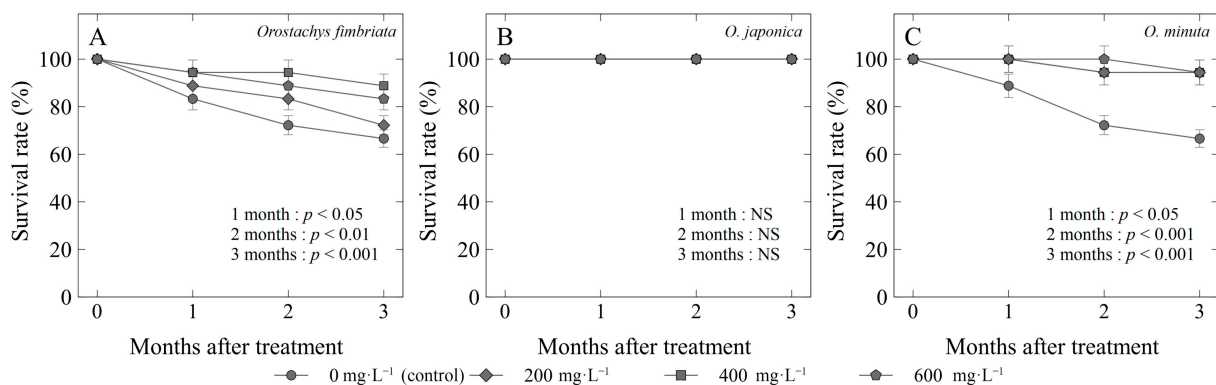


Figure 3. Survival rate (%) of three *Orostachys* species: (A) *O. fimbriata*; (B) *O. japonica*; and (C) *O. minuta* offsets treated with different concentrations of gibberellic acid (GA_3). Non-significant (NS) or significant at $p < 0.05$, 0.01 , or 0.001 .

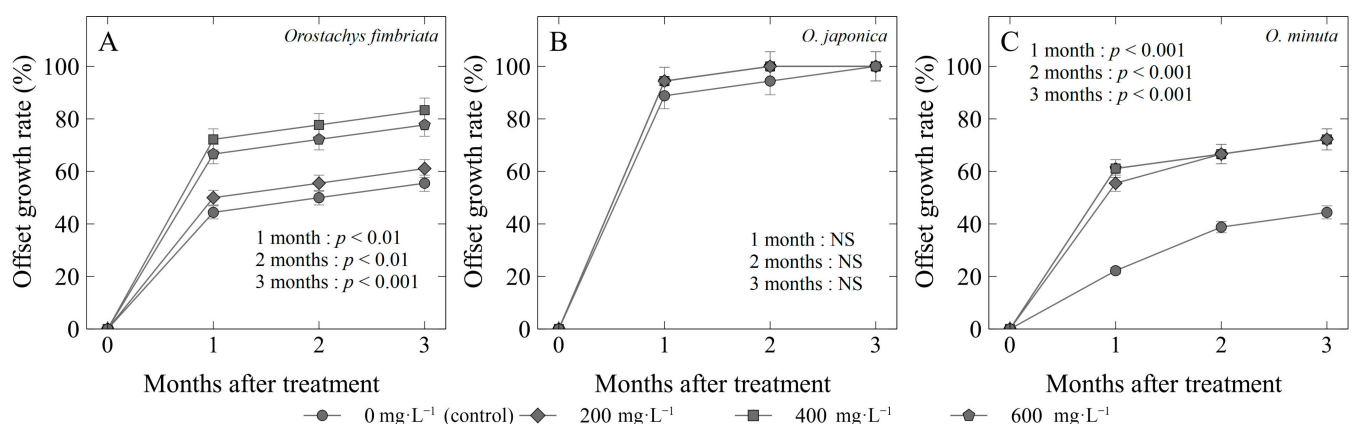


Figure 4. Offset growth rate (%) of three *Orostachys* species: (A) *O. fimbriata*; (B) *O. japonica*; and (C) *O. minuta* offsets treated with different concentrations of gibberellic acid (GA_3). Non-significant (NS) or significant at $p < 0.01$ or 0.001 .

The offset growth rate of *Orostachys* species was determined for three consecutive months (Figure 4). For *O. fimbriata*, the use of 400 mg·L⁻¹ (83%) on the offsets gave the highest growth rate, while the control gained the lowest value of 55% (Figure 4A). Similar to the survival rate, *O. japonica* with applied GA₃ did not have any significant differences with those of the control from the first up to the third month (Figure 4B). However, it may be noted that the earliest and highest offset growth rate at 1 month (94%) and 2 months (100%) after treatment was taken from those treated with GA₃ compared with the control (88% at 1 month, 94% at 2 months). The application of gibberellic acid gave the significantly highest offset growth in *O. minuta* species for the three consecutive months, with 72% (200 and 400 mg·L⁻¹) to 83% (600 mg·L⁻¹), while the control lagged at 44%.

3.2. Plant Growth Parameters

Aside from the survival rate and growth rate of *Orostachys* species offsets, the application of GA₃ also significantly affected the growth and development parameters of these succulents (Table 1).

Table 1. Plant growth parameters of *Orostachys* species in response to gibberellic acid (GA₃) concentrations.

Species	Treatments (mg·L ⁻¹)	Plant Sizes (cm)				Offset Parameters		Leaf Sizes (cm)		Ground Cover (cm ²)
		Shoot Height	Shoot Width	Stem Diameter	Root Length	No. of Offsets	Length (cm)	Length	Width	
<i>O. fimbriata</i>	0 (Control)	3.15 ± 0.17 ^z b _y	3.77 ± 0.38 b	0.24 ± 0.01 c	5.03 ± 0.75 a	16.0 ± 0.61 a	3.29 ± 0.94 a	2.58 ± 0.10 d	0.50 ± 0.06 b	14.9 ± 1.89 c
	200	4.71 ± 0.31 a	5.19 ± 0.50 a	0.27 ± 0.02 bc	4.78 ± 0.40 a	18.0 ± 1.54 a	1.59 ± 0.48 a	2.88 ± 0.18 c	0.48 ± 0.09 b	26.2 ± 2.86 b
	400	5.08 ± 0.26 a	5.58 ± 0.48 a	0.31 ± 0.01 b	3.93 ± 0.67 a	19.6 ± 1.34 a	1.87 ± 0.55 a	3.43 ± 0.12 b	0.54 ± 0.07 b	32.0 ± 2.59 a
	600	5.31 ± 0.21 a	6.04 ± 0.53 a	0.35 ± 0.01 a	5.46 ± 0.59 a	19.6 ± 1.76 a	2.19 ± 0.87 a	3.87 ± 0.14 a	0.60 ± 0.05 a	38.0 ± 3.05 a
Significance ^x		**	**	**	NS	NS	NS	**	**	**
<i>O. japonica</i>	0 (Control)	3.73 ± 0.13 b	4.83 ± 0.28 a	0.37 ± 0.02 a	6.36 ± 0.38 a	9.5 ± 0.87 a	1.98 ± 0.11 b	3.08 ± 0.23 a	0.60 ± 0.00 a	23.7 ± 1.93 a
	200	6.65 ± 0.16 a	4.75 ± 0.22 a	0.37 ± 0.03 a	5.78 ± 0.26 ab	8.3 ± 0.82 a	3.73 ± 0.16 a	3.54 ± 0.15 a	0.63 ± 0.01 a	23.0 ± 1.58 a
	400	6.52 ± 0.30 a	4.82 ± 0.19 a	0.41 ± 0.01 a	4.69 ± 0.19 b	9.3 ± 1.11 a	3.87 ± 0.20 a	3.27 ± 0.20 a	0.63 ± 0.02 a	23.6 ± 1.43 a
	600	6.67 ± 0.24 a	4.54 ± 0.38 a	0.40 ± 0.01 a	5.50 ± 0.28 ab	8.8 ± 0.75 a	3.23 ± 0.15 a	3.42 ± 0.25 a	0.64 ± 0.02 a	21.3 ± 2.56 a
Significance		**	NS	NS	*	NS	**	NS	NS	NS
<i>O. minuta</i>	0 (Control)	1.88 ± 0.21 c	2.66 ± 0.32 b	0.22 ± 0.01 d	4.40 ± 0.66 a	8.7 ± 0.97 c	0.78 ± 0.16 c	1.43 ± 0.12 c	0.40 ± 0.02 c	8.6 ± 2.34 d
	200	3.42 ± 0.24 b	4.17 ± 0.22 a	0.27 ± 0.01 c	5.31 ± 0.42 a	16.8 ± 1.64 ab	1.62 ± 0.19 b	2.44 ± 0.21 b	0.44 ± 0.02 b	17.8 ± 1.66 c
	400	3.83 ± 0.18 b	4.96 ± 0.24 a	0.30 ± 0.01 b	5.15 ± 0.38 a	20.5 ± 1.84 a	1.99 ± 0.12 b	3.16 ± 0.28 ab	0.48 ± 0.03 b	25.1 ± 1.85 b
	600	5.15 ± 0.29 a	5.18 ± 0.27 a	0.37 ± 0.01 a	4.78 ± 0.52 a	13.6 ± 2.36 b	2.42 ± 0.17 a	3.62 ± 0.26 a	0.53 ± 0.02 a	27.7 ± 2.03 a
Significance		**	**	**	NS	**	**	**	**	**

^z Mean ± standard error (SE). ^y Significant differences ($p < 0.05$) among columns with different letters were analyzed using Duncan's multiple range test; same letters indicate no significant difference. ^x Non-significant (NS) or significant at $p < 0.05$ (one asterisk; *) or 0.01 (two asterisks; **).

The use of GA₃ significantly affected the shoot height in all *Orostachys* species, wherein all concentrations significantly differ from those of the control. The use of 600 mg·L⁻¹ had the highest value for *O. fimbriata* (5.31 cm) and *O. japonica* (6.67 cm), which did not significantly differ from other concentrations. However, among *O. minuta*, the application of 600 mg·L⁻¹ GA₃ (5.15 cm) differed significantly from those of 200–400 mg·L⁻¹ (3.42–3.83 cm) and of the control (1.88 cm).

Likewise, the shoot width of *O. fimbriata* and *O. minuta* treated with GA₃ at different concentrations was significantly wider than that of the control. Similarly, the stem diameter of *O. fimbriata* and *O. minuta* was significantly affected by treatment application, wherein the use of 600 mg·L⁻¹ gave the widest diameter, which was different from the other concentrations.

In terms of root growth, *O. fimbriata* and *O. minuta* were not significantly affected by GA₃ application. On the other hand, control plants of *O. japonica* species had the longest root length of 6.36 cm compared to those treated with gibberellic acid.

For *O. minuta*, the use of 400 mg·L⁻¹ GA₃ produced the highest number of offsets with 20.5, compared to those of 200, 600 mg·L⁻¹ (16.8, 13.6 offsets, respectively), and the control (8.7 offsets). In the case of *O. japonica*, the offset length was found to be highest in GA₃ treatments (3.23–3.73 cm) regardless of the concentration. Nevertheless, for *O. minuta*, the use of the highest concentration (600 mg·L⁻¹) resulted in the highest offset length of 2.42 cm, which significantly differed from other treatments.

The application of $600 \text{ mg}\cdot\text{L}^{-1}$ gave the significantly highest leaf length and width in *O. fimbriata* (3.87 cm, 0.60 cm) and *O. minuta* (3.62 cm, 0.53 cm), which significantly differed from those of other treatment concentrations and the control. The results of the shoot and root growth of *Orostachys* species are evident in the representative samples shown in Figure 5.

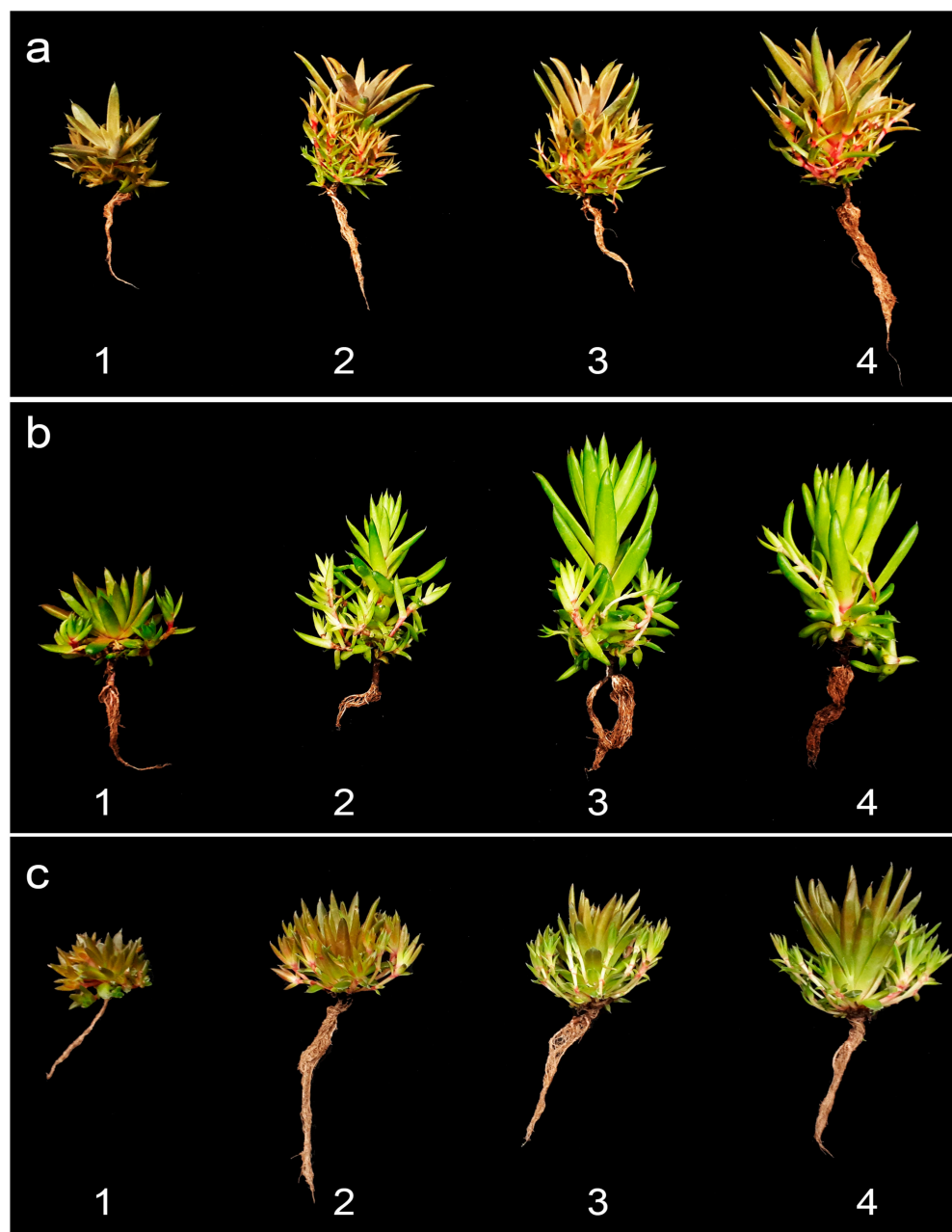


Figure 5. Comparison of growth and development of *Orostachys* species: (a) *O. fimbriata*; (b) *O. japonica*; and (c) *O. minuta* of the (1) control and those treated with gibberellic acid (GA_3) at different concentrations: (2) $200 \text{ mg}\cdot\text{L}^{-1}$; (3) $400 \text{ mg}\cdot\text{L}^{-1}$; and (4) $600 \text{ mg}\cdot\text{L}^{-1}$.

With regards to the ground cover, the application of $600 \text{ mg}\cdot\text{L}^{-1}$ resulted in the greatest ground cover in *O. fimbriata* (38.0 cm^2) and *O. minuta* (27.7 cm^2). However, in *O. japonica*, the ground cover showed no significant differences by treatment.

3.3. Moisture Content

Determining the moisture content is deemed crucial and provides a good overview of the overall growth and development performance of offsets concerning their nutrient uptake, water regulation, and plant vigor. The fresh and dry weight of *Orostachys* species were gathered to further determine their moisture content (Table 2). The results of the analysis revealed that the moisture content and dry and fresh weight of *O. fimbriata* and *O. minuta* were significantly affected by gibberellic acid application, except for the root moisture content. However, for *O. japonica*, no significant differences between the treatments were recognized.

Table 2. Fresh and dry weight and moisture content of *Orostachys* species in response to gibberellic acid (GA₃) concentrations.

Species	Treatments (mg·L ⁻¹)	Fresh Weight (g)		Dry Weight (g)		Moisture Content (%)	
		Shoot	Root	Shoot	Root	Shoot	Root
<i>O. fimbriata</i>	0 (Control)	3.06 ± 0.23 ^z d ^y	0.03 ± 0.00 d	0.17 ± 0.02 b	0.01 ± 0.00 b	94.3 ± 0.26 a	72.2 ± 1.11 a
	200	3.85 ± 0.28 c	0.04 ± 0.00 c	0.19 ± 0.02 b	0.01 ± 0.00 b	94.9 ± 0.15 a	73.8 ± 2.49 a
	400	5.05 ± 0.40 b	0.05 ± 0.01 b	0.36 ± 0.06 b	0.01 ± 0.00 b	93.3 ± 0.56 a	74.7 ± 2.12 a
	600	6.44 ± 0.51 a	0.07 ± 0.01 a	0.65 ± 0.12 a	0.02 ± 0.01 a	90.4 ± 0.68 b	75.5 ± 1.30 a
Significance ^x		**	**	**	**	**	NS
<i>O. japonica</i>	0 (Control)	7.66 ± 0.97 a	0.18 ± 0.02 a	0.55 ± 0.09 a	0.04 ± 0.00 a	92.7 ± 0.29 a	75.9 ± 1.68 a
	200	10.21 ± 0.89 a	0.18 ± 0.01 a	0.64 ± 0.10 a	0.04 ± 0.01 a	93.8 ± 0.38 a	75.6 ± 0.72 a
	400	10.03 ± 1.39 a	0.17 ± 0.02 a	0.64 ± 0.12 a	0.04 ± 0.00 a	93.7 ± 0.23 a	77.5 ± 1.25 a
	600	10.37 ± 1.07 a	0.14 ± 0.03 a	0.85 ± 0.18 a	0.03 ± 0.01 a	92.3 ± 0.85 a	77.1 ± 0.87 a
Significance		NS	NS	NS	NS	NS	NS
<i>O. minuta</i>	0 (Control)	1.83 ± 0.34 d	0.03 ± 0.01 c	0.10 ± 0.03 b	0.01 ± 0.00 b	92.8 ± 0.60 b	73.4 ± 2.57 a
	200	4.32 ± 0.42 c	0.07 ± 0.01 ab	0.26 ± 0.04 b	0.02 ± 0.00 a	94.0 ± 0.13 a	74.5 ± 1.88 a
	400	5.40 ± 0.36 b	0.06 ± 0.00 b	0.30 ± 0.03 b	0.01 ± 0.00 b	94.5 ± 0.12 a	77.1 ± 1.11 a
	600	7.23 ± 0.65 a	0.09 ± 0.01 a	0.67 ± 0.10 a	0.02 ± 0.00 a	91.2 ± 0.78 b	76.0 ± 1.35 a
Significance		**	**	**	**	**	NS

^z Mean ± SE. ^y Significant differences ($p < 0.05$) among columns with different letters were analyzed using Duncan's multiple range test; same letters indicate no significant difference. ^x Non-significant (NS) or significant at $p < 0.01$ (two asterisks; **).

The results showed that the use of 600 mg·L⁻¹ GA₃ exhibited a significantly higher fresh and dry weight of roots and shoots of *O. fimbriata* offsets compared to other concentrations and the control. Interestingly, the moisture content was observed to be higher in those treated with 200 mg·L⁻¹ (94.9%), which did not significantly differ from those of the control (94.3%) and 400 mg·L⁻¹ (93.3%).

Similar results were found to those of *O. minuta* offsets, wherein the 600 mg·L⁻¹ treatment resulted in the heaviest shoot (7.23 g) and root (0.09 g) fresh weight. The heaviest shoot dry weight was found in those treated with 600 mg·L⁻¹, which significantly differed from all other treatments; however, for the root dry weight, the application of both 200 mg·L⁻¹ and 600 mg·L⁻¹ gave the highest root dry weight of 0.02 g. The highest shoot content was finally found in those treated with 400 and 200 mg·L⁻¹, with 94.5% and 94.0%, respectively.

4. Discussion

The dormant axillary buds of three *Orostachys* spp. were treated with various concentrations of gibberellic acid to determine its efficacy in inducing growth and development for better plant propagation methods of these economically valuable succulent plants. The use of GA in plant propagation, especially among horticulturally valued plants, has been a common practice, as it has impactful results at low concentrations and is easily handled by nursery propagators [18,34]. However, it was always recommended that trials on its concentration should be performed, as these exogenous hormones have varying effects on different plant species, wherein it could lead to inhibitory effects such as dwarfism in some cases [35]. Hence, this study was deemed necessary.

The results revealed that the use of GA₃ had a significantly higher survival rate in *O. fimbriata* and *O. minuta* species compared to the controls. Despite the common use of gibberellins for shoot development, it has also been reported to promote tissue callus development [36] and increase cell division and elongation [37], which are important

mechanisms for plant propagule survival, especially of those that are vegetatively propagated such as suckers, offsets, runners, and plant cuttings [18]. Callus formation, the development of unorganized cell masses, has profound importance in the propagation of horticultural plants, as it prevents further infection and water loss [38–40] and is often suggested to be highly pluripotent or associated with the regeneration of new organs and tissues [41]. Efferth [42] reported that callus formation may be driven by the application of plant hormones, including gibberellins.

On the other hand, *O. japonica* showed a 100% survival rate regardless of the treatment. This result has also been found in some plants wherein GA₃ did not affect plant regeneration, such as those in the study of Tamaki et al. [43] on *Graptopetalum paraguayense* succulents. According to Kato [44], gibberellins are sometimes unable to stimulate cells in this regard, which would still warrant further investigation of its mechanism.

The offset growth rate of the majority of the *Orostachys* species was also significantly affected by the exogenous application of gibberellins, regardless of the concentration, which significantly differed from those of the controls. Several crops have been reported to show positive results in enhancing the growth of dormant propagules of asexually propagated crops such as *Acer* and *Prunus* hardwood cuttings [45], *Gladiolus* corms [46], *Picrorhiza kurroa* rooting runner cuttings [47], garlic bulbs [48], globe artichoke [49], and even seed-bearing plants such as *Panax notoginseng* [50], *Vigna mungo* [51], and papaya [52], which are usually perpetuated using mucilage, cold conditions, low light intensities, etc. The use of GA₃ on plant propagules causes an alteration of the hormone balance, thus inducing hydrolytic enzyme formation, which regulates the mobilization of reserved food resources [18,46,53]. It has also been shown that GA-promoted growth may mediate these food reserves through the accumulation of soluble sugars, starch, and cell wall polysaccharides [54]. Aside from the mobilization of food, GA₃ has also been associated with antagonistic interactions with abscisic acid, which induces dormancy and lagged growth; however, the exogenous application of GAs counters it [27]. Likewise, the study of Beauvieux et al. [55] reported that when GA₃ is applied, it could indirectly promote vegetative development through the stimulation of oxidative stress responses, which was found to increase the production of proteins involved in oxidative reduction responses by 40% in Japanese apricots [56]. These mechanisms coherently work to enhance the survival rate and give a higher offset growth rate in *O. fimbriata* and *O. japonica*.

In terms of shoot growth parameters, the use of GA₃, especially at the highest concentration, resulted in the tallest and widest plants with long stem lengths. Our results are similar to the numerous studies that have been conducted showing gibberellin as an effective plant growth enhancer of shoot development and plant production in general, especially for horticulture and viticulture [57]. The most prominent effect of gibberellins is their role in internode elongation [58]. Due to their first discovery in rice, stem-promoting gibberellins have been studied and applied in other crops to induce bolting and leaf growth [59]. GA's action targets the intercalary meristem, where cell division and elongation activities are increased [22,60].

Similarly, the root growth parameters were also found to be higher in those treated with GA₃ than those of the control, but the differences between treatments were only significant in two species, i.e., *O. japonica* and *O. minuta*. Although several papers suggest that GA plays a minimal and less remarkable role, sometimes even detrimental, in root functions and development [60–62], studies by Bidadi et al. [63] suggest that there just needs to be a proper balance between the production of bioactive GAs in the root to promote root growth. Likewise, Binenbaum et al. [64] emphasized that GA movement is deemed necessary at multiple stages of development in all plant organs, such as the local accumulation of bioactive GA in the root elongation zone, because it is directly correlated with cellular growth. Other vegetatively propagated plants have also reported root growth with the application of GA, such as *Pennisetum purpureum* [65], *Lemna minor* [66], and sugar beets [67], which had similar results to those of *Orostachys* species.

Gibberellin has been found to support leaf expansion [68,69], which would explain the leaf length, width, and ground coverage of *Orostachys* species. The use of GA has been prominent in ground cover crops [70–72] and other ornamentals [73–75] to increase leaf growth, thus increasing ground coverage. In the study of Nelissen et al. [76], this leaf expansion was suggested to be caused when there is maximum GA in the division zone (DZ). When altering the GA levels, it would also have a definitive effect on the DZ size, resulting in proportional growth or a change in organ growth rates.

The moisture content of *Orostachys* species treated with GA₃ had only significant differences in *O. fimbriata* and *O. minuta*. The results suggest that the majority of GA-treated offsets had an increased moisture content than those of untreated offsets. GA, being a key plant hormone, has a vital role in regulating signal pathways that help plants adapt to several abiotic stress conditions and promote plant growth, including water uptake [18,77]. The accompanying leaf expansion and shoot growth prompted by the application of GA as a signaling hormone that promoted water uptake sheds light on the increased moisture content of treated *Orostachys* plants. Studies of several vegetatively propagated plants have shown increased rates of shoot and propagation rates when treated with GA₃ [78–80].

5. Conclusions

Offsets of *Orostachys* species, particularly *O. fimbriata* and *O. minuta*, induced with gibberellic acid (GA₃) had significantly higher and earlier offset growth rates. Through the evaluation of their offset growth and development, significant differences in shoot and root growth, including moisture content, were observed, which would allow faster and more efficient vegetative propagation rates for this economically important succulent throughout the year, even during the cold winter months where slow growth is prevalent.

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