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Assessing the Impact of Plant Hormones on *Osmanthus* spp. Cutting Propagation

Yulong Chen[1], Julia Rycyna [1,2] and Ping Yu [1]

[1]Department of Horticulture, University of Georgia, Griffin, Georgia 30223 USA

[2]Environmental Horticulture Department, University of Florida, Gainesville, Florida 32607 USA

Yulong.Chen@uga.edu

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Summary

Osmanthus is a genus of ornamental plants with valuable qualities such as pest resistance, evergreen foliage, and aromatic flowers. However, different species respond differently to plant hormones in regard to propagation success. This study evaluated five different plant hormones/rates (3,000 mg/L of indole-3-butyric acid (IBA) powder, 500 mg/L and 2,000 mg/L of potassium indole-3-butyric acid (K-IBA) solution, 10% seaweed extract solution effects on six different *Osmanthus* species (*Osmanthus heterophyllus* ‘Kaori Hime’, *Osmanthus armatus* ‘Jim Porter’, *Osmanthus* × *fortunei* ‘Patty's Secret’, *Osmanthus heterophyllus* ‘Rotundifolius’, *Osmanthus delavayi, and Osmanthus* × *fortunei* ‘Fruitlandii’)cuttings. The water dip was used as the control. Cuttings’ rooting rates, root length, and survival rate were measured. The results showed the *Osmanthus heterophyllus* ‘Kaori Hime’ exhibited the highest rooting rate, root length, and survival rate, while the *Osmanthus heterophyllus* ‘Rotundifolius’ had the lowest survival rate. The species significantly influence the rooting percentage, root length and survival.

INTRODUCTION

Osmanthus, a genus consisting of about 30 species, mostly native to Eastern and southeastern Asia (Qian et al., 2023), is widespread across several Asian countries, including Japan, Korea, southwestern China - the Himalayas, and North America (Wang et al., 2022). *Osmanthus* has a wide range of growth habits, is planted in landscapes, and is adaptable to different environmental conditions.

Stem cuttings are the most commonly used asexual propagation method for woody plants (Nair et al., 2008). For a successful stem cutting propagation, different hormones were used to promote rooting rate, root length, and uniformity (Lewis et al., 2020). For instance, IBA (Indole-3-butyric acid) at 2,500 mg/L was used to improve rooting of *O. heterophyllus* 'Ilicifolius' hardwood cuttings from 80.6% (the control) to 86.1% (Blazich and Acedo, 1989). Additionally, hardwood cuttings of *Asclepias tuberosa* (Butterfly weed) treated with K-IBA (Potassium Indole-3-butyric acid) at 3000mg/L had greater root quality (11.9 ± 1.4) compared to the control (9.2 ± 1.3) (Lewis et al., 2020).

Seaweed extract Kelpak®, made from the kelp *Ecklonia maxima*, contains several phytohormones, including auxins, cytokinins, gibberellins, and abscisic acid (Stirk et al., 2014). It has been widely used by agriculture to enhance plant health and rooting rate (Stirk et al., 2014). For instance, using 10% Kelpak® has increased the rooting rate of hybrid tea rose (*Rosa* × *hybrida*) stem cuttings by 11% compared to the control (distilled water) (Traversari et al., 2022).

Additionally, different species (even the types of stem cutting) of *Osmanthus* respond to hormones differently. For instance, semi-hardwood cuttings of *O. heterophyllus* 'Ilicifolius' (>80%) had a significantly higher rooting rate compared to *O. heterophyllus* 'Rotundifolius' (0%). In the same study, 2,500mg/L IBA significantly increased the rooting of softwood cuttings for *O.* × *fortunei* from 63.9% (control) to 91.7% (Blazich and Acedo, 1989). However, research on different species of *Osmanthus* response to hormones is still insufficient, and there are few relevant studies on *O. delavayi* and *O. armatus*. Thus, this study aims to provide insights into the different *Osmanthus* species propagation responses to hormones.

MATERIALS AND METHODS

Six *Osmanthus* species including *O. heterophyllus* 'Kaori Hime', *O. armatus* 'Jim Porter', *O.* × *fortunei* 'Patty's Secret', *O. heterophyllus* 'Rotundifolius', *O. delavayi*, and *O.* × *fortunei* 'Fruitlandii' were used in this study. All the cuttings were semi-hardwood cuttings obtained on 16 March 2023, from Atlanta Botanical Garden (Gainesville, GA, USA). Stems were cut into 2-in (5.1 cm) cuttings with the top 1 or 2 leaves remaining and treated with three hormones: indole-3-butyric acid (IBA) powder (Hormodin® 2, at 3,000 mg/L), potassium indole-3-butyric acid (K-IBA) at 500 mg/L and 2,000 mg/L, and Kelpak® at a 10% solution; the water dip treatments were used as the control. For the IBA powder treatment, the basal ends of the cuttings were first moistened to facilitate adherence to the powder and then dipped into the IBA powder to ensure comprehensive coverage. The excess powder was removed by lightly tapping the cuttings. For liquid formulations treatments (K-IBA, Kelpak®, and water), the cuttings basal (2 cm) were immersed for

5 seconds and air dried for at least 10 minutes before being inserted in the media.

Cuttings were inserted into 72-cell plug trays (21.25×11×2.25 inches) filled with 7:3 (by vol.) peat to perlite media. The media components used in this study included peat (The Gold Canadian Sphagnum Peat Moss; Voluntary Purchasing Groups, Inc., Fort Worth, TX, USA) to perlite (Dicaperl Hydraged Aluminum by Dicalite Management Group; Dicalite Management Group, Bala Cynwyd, PA, USA). Rooting percentage and root length (cm) were measured on April 26 (week 6). The rooted cuttings were then transplanted into square pots (1½ inches square by 2¼ inches deep) filled with Jolly Gardener Pro-line C/25 Growing Mix (Jolly Gardener; Oldcastle Lawn & Garden Inc., Atlanta, GA, USA). Survival rates were recorded on April 26 (week 6), May 16 (week 9), and June 6 (week 12) after treatments.

The cuttings were placed in a glass-covered greenhouse on the University of Georgia, Griffin campus. During the experiment, day and night temperatures were recorded at 29.3±2.8°C and 20.8±1.0°C, respectively. Relative humidity high and low levels were approximately 90.54±0.97 and 52.70±2.06%. Intermittent mist operated 4 seconds every 6 minutes daily.

The experimental design was a complete randomized design, each experimental unit consists of a single treatment applied to an individual species, with 12 replicates. Data were analyzed with One-way and Two-way Analysis of Variance using R program software (version 4.3.1; RStudio, Boston, MA, USA) to test the effect of different levels of hormones and different species on the rooting rate, average root length, and survival rate. Duncan's multiple range tests were used to compare means among treatments at $P < 0.05$.

RESULTS

There were no two-factor interactions for root length or survival rates, while the interaction between species and treatment significantly impacted the rooting rate (**Table 1**). The hormone did not significantly affect the rooting rate, root length, or survival rate. Conversely, the *Osmanthus* species significantly influenced the rooting percentage, root length, and survival rate.

Table 1. ANOVA of cuttings rooting rate, root length, and survival rate as influenced by hormone treatments and *Osmanthus* species.

Source	Rooting rate (%)	Root length (cm)	Survival rate (%)
Hormone	NS	NS	NS
Species	*	***	***
Hormone × Species	*	NS	NS

[z] *, **, *** show significant difference at $P \leq 0.05$, 0.01 and 0.001 respectively; NS, not significant at $P > 0.05$.

Table 2. The influence of *Osmanthus* species on the rooting rate, root length, and survival rate of cuttings.

Species	Rooting rate (%) [z, y]	Root length (cm) [z, y]	Survival rate (%) [z]		
			Week 6	Week 9	Week 12
O. heterophyllus 'Kaori Hime'	21.7±5.4a	3.950±1.242a	86.7±4.2a	78.3±5.7a	68.3±10.3a
O. armatus 'Jim Porter'	0.0±0.0b	0.0±0.0b	80.0±4.2a	56.7±7.2b	35.0±3.1bc
O. × *fortunei* 'Patty's Secret'	3.3±2.3b	0.333±0.235b	58.3±7.9b	36.7±8.6bc	20.0±5.7c
O. heterophyllus 'Rotundifolius'	——	——	0.0±0.0d	0.0±0.0d	0.0±0.0d
O. delavayi	1.7±1.7b	0.017±0.017b	41.7±7.9c	28.3±3.3c	21.7±4.2bc
O. × *fortunei* 'Fruitlandii'	6.7±3.2b	1.600±1.294b	70.0±5.0ab	53.3±10.1b	38.3±5.7b

[z] Each value is based on 60 cuttings.
[y] Each value is based on the number of cuttings rooted for a particular treatment.
[x] Measurements of survival rate were taken on April 26, 2023 (week 6), May 16, 2023 (week 9), and June 6, 2023 (week 12). The rooting rate and root length were measured on April 26, 2023 (week 6).
[w] Data are shown as Mean ± SE. Different letters indicate significant differences among the species at $p < 0.05$.

Different species significantly impacted the rooting rate, root length, and survival rates (**Table 2**). The *O. heterophyllus* 'Kaori Hime' had a significantly higher rooting rate (21.7 ± 5.7%) and longer root length (4.0±1.2cm) than the other species. The rate and length of *O.* × *fortunei* 'Patty's Secret' (3.3±2.3% and 0.3±0.2cm), *O.* delavayi (1.7±1.7% and 0.02±0.02 cm), and *O.* × *fortunei* 'Fruitlandii' (6.7±3.2% and 1.6±1.3 cm) were significantly lower than that of *O. heterophyllus* 'Kaori Hime'. *O. heterophyllus* 'Kaori Hime' consistently exhibited significantly higher survival rates than other species on weeks 6, 9, and 12, which was 86.7±4.2%, 78.3±5.7%, and 68.3±10.3%, respectively. *O.* delavayi showed the second-lowest survival rate in week 6 (41.7±7.9%) and week 9 (28.3±3.3%). While in week 12, *O.* × *fortunei* 'Patty's Secret' had the second-lowest survival rate, at 20.0±5.7%. In week 6, all the *O. heterophyllus* 'Rotundifolius' were decreased, and *O. armatus* 'Jim Porter' did not produce any roots.

Although different hormones did not significantly impact the rooting rate, root length, or survival rate (**Table 3**), the cuttings treated by the 500 mg/L K-IBA had the highest rooting rate (11.7±4.2%) and root length (2.4±1.4 cm) among all the hormones used in this study. The cuttings treated with 3,000 mg/L IBA powder, 10% Kelpak, and the control exhibited the lowest rooting (5.0±2.8%), with the 3,000 mg/L IBA powder treatment having the shortest root length (0.3±0.2 cm).

Table 3. The influence of the types and rates of plant growth hormones on the rooting rate, root length, and survival rate of cuttings.

Treatment	Rooting rate (%) [z, y]	Root length (cm) [z, y]	Survival rate (%) [z]		
			Week 6	Week 9	Week 12
3,000 mg/L IBA powder	5.0±2.8a	0.300±0.231a	59.7±13.5a	52.8±13.6a	29.2±7.7a
500 mg/L K-IBA	11.7±4.2a	2.433±1.382a	47.2±13.7a	34.7±11.9a	29.2±11.5a
2,000 mg/L K-IBA	6.7±3.2a	1.783±0.957a	61.1±13.9a	40.2±13.7a	30.5±14.7a
10% Kelpak	5.0±2.8a	1.067±0.697a	55.6±14.7a	37.5±11.5a	31.9±10.0a
Control (water dip)	5.0±2.8a	0.317±0.204a	56.9±12.8a	45.8±10.0a	31.9±7.9a

[z] Each value is based on 72 cuttings.
[y] Each value is based on the number of cuttings which rooted for a particular treatment.
[x] Measurements of survival rate were taken on April 26, 2023 (week 6), May 16, 2023 (week 9), and June 6, 2023 (week 12). The rooting rate and root length were measured on April 26, 2023 (week 6).
[w] Data are shown as Mean ± SE. Different letters indicate significant differences among the species at *P*< 0.05.

DISCUSSION

At week 6 there was complete mortality of *O. heterophyllus* 'Rotundifolius' semi-hardwood cuttings – which failed to root. However, Blazich and Acedo (1989) reported that hardwood cuttings of 'Rotundifolius' rooted in high percentages without auxin. Seasonal timing could be a key factor in the rooting of this cultivar. The lack of success of semi-hardwood cuttings in our study align with Faust et al. (2016) who attributed cutting failure in different species to difficulties in root initiation and susceptibility to environmental stress during propagation. (Faust et al., 2016).

Moreover, this study found no significant difference between chemical treatments and the control on rooting rate, root length, and survival rate. The results can be attributed to the seasonal effects on the efficacy of hormone treatments (the experiment lasted from March 16 to June 6, 2023) – and differences among cutting types; hardwood and softwood cuttings were not tested. According to Southworth and Dirr (1996), March to June is less conducive for K-IBA treatment in promoting the rooting rate of *Cephalotaxus harringtonia* (Japanese plum-yew) cuttings. This suggests that environmental factors like temperature, humidity, and light conditions play a crucial role in plant propagation success and may even overshadow the influence of hormonal treatments.

The genotype and growth regulators are the main factors influencing rooting rate and root length in cuttings (Metaxas et al., 2004). In this study, we found that the different *Osmanthus* species had a significantly higher impact on the rooting rate, root length, and survival rate of cuttings than hormones. The results align with the findings of Gomes et al. (2010), who reported that when considering the number of roots formed per explant, factors such as the genotype and the periods of root induction and development have a significant influence.

Similarly, Oboho and Iyadi's study showed that different species treated with water dip had different survival rates with *Garcinia kola* (bitter kola) having the highest, at 85%, followed by *Gambeya albida* (white star apple) at 75%, *Irvingia gabonensis* (wild mango) at 25%, *Annona muricata* (soursop) at 15%, and *Triplochiton scleroxylon* (African whitewood) at 0% (Oboho and Iyadi, 2013).

CONCLUSION

In conclusion, the chemical treatments of auxins and Kelpak® -seaweed extract - did not influence rooting. However, different species responded significantly differently in rooting percentage, root length, and survival rate. Future research should cover a broader range of species, different environmental conditions, seasonal variation with different cutting types, and different types and rates of rooting hormones.

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