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Different Responses to Adventitious Rhizogenesis under Indole-3-Butyric Acid and Seaweed Extracts in Ornamental's Cuttings: First Results in *Photinia x fraseri* 'Red Robin'

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Abstract: Fraser's photinia 'Red Robin' (*Photinia x fraseri* Dress, *Rosaceae* family) is an important primary ornamental landscaping species with optimal hedge or screen effects and low maintenance, but it is difficult to root when propagated by cuttings, although high concentrations of phytohormones are used to optimize rhizogenesis. To our knowledge, there is currently no feasible enhanced method for photinia vegetative propagation through stem cuttings, using seaweed extract-based biostimulants as root promoters. Given the economic importance of the species, this research aims to assess the effects of indole-3-butyric acid (IBA) and seaweed extract-based stimulators on the quality of photinia 'Red Robin' cuttings, in terms of rooting indicators and ground and aboveground agronomic features. The treatments applied were different concentrations of commercial rooting stimulators compared to an untreated control: C0: distilled water; Rhizopon AA: 1% IBA (R1); Kelpak®: 2 mL L^{-1} (K2); Kelpak®: 3 mL L^{-1} (K3); Goteo®: 2 mL L^{-1} (G2); Goteo®: 3 mL L^{-1} (G3). The first results showed different responses to adventitious rhizogenesis under IBA and both seaweed extract treatments. At 70 DAC (days after cutting), the seaweed extract stimulated the production of over 80% of cuttings with callus; at 240 DAC, the percentage of rooted cuttings treated under R1 was the highest = 34.3%; the worst results were obtained by both biostimulant treatments at the highest doses: K3 = 21.3% and G3 = 20.7%. Furthermore, R1 produced 3.07 roots per cutting, which was 50% higher than the average of all other treatments. The applications of Kelpak® and Goteo® biostimulants, at both concentrations, resulted in an inhibition of root length with values below the untreated control. Rooted cuttings under R1 showed the highest ground (0.35 g) and aboveground (0.47) dry value. Neither seaweed extract, Kelpak® or Goteo®, at different concentrations, improved both the ground and above-ground weights of rooted cutting, compared to the untreated control, indicating that these natural products are not suitable for Fraser's photinia 'Red Robin' propagation using this methodology. The overall quality of cuttings in IBA treatment was the strongest, with 1%, being the optimum concentration. Further research must be conducted to propose effective agronomic protocols by investigating application methods, doses and number of applications, and to clarify the biochemical and molecular mechanisms of action of these seaweed extracts.

Keywords: *Ascophyllum nodosum*; auxin; biostimulants; cutting; *Ecklonia maxima*; ornamental plants; quality rooted cutting; root architecture; vegetative propagation

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1. Introduction

Nowadays, one of the critical challenges to address is increasing global agricultural production and crop quality, through reducing the impact of agriculture on ecosystems [1,2]. Increasing the quality and yield of crops, maintaining agroecosystems, reducing chemical substances, increasing nutrient uptake and use efficiencies, and stimulating the plant's natural defense, must be the major goals [3–5]. The quality of propagation material is one of the most important factors for increasing the productivity of any ornamental crop.

Several authors have dealt with defining the quality of the propagation material from a genetic, sanitary and agronomic point of view. From a genetic point of view, quality is related to the endogenous content of auxins [6]. Sanitary quality is characterized by the absence of parasites and pathogens [7]; the agronomic quality of a rooted cutting is based on the morphological features of the aerial parts and adventitious roots, which influence health, vigor and uniformity [8,9]. A high number of very fine roots, which are essential for continuous access to water and nutrients, can help the plant withstand transplant shock, increasing survival and plant growth [10]. A multitude of internal and environmental factors affect the production of adventitious roots. Among the internal components, phytohormones, particularly auxins, have the most significant impact. Natural (indole-3-butyric acid, IBA) and synthetic auxins (naphthalene acetic acid, NAA), applied exogenously to cuttings [11], play a key role in generating adventitious roots [12,13] and balanced shoots in ornamental species that are recalcitrant to clonal propagation, due to a low concentration of endogenous auxins [10,14–22]. Commercial products based on indole-3-butyric acid (IBA), such as Rhizopon, are one of the most widely used exogenous sources of auxin and they can be delivered to cuttings in talc or dissolved in alcohol to be used as a quick dip [23]. The most recent literature has expanded the research on other natural substances with rooting activity such as biostimulants [24–26]. Biostimulants are defined as "product stimulating plant nutrition processes, independently of the product's nutrient content, with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: (a) nutrient use efficiency; (b) tolerance to abiotic stress; (c) quality traits; (d) availability of confined nutrients in soil or rhizosphere" [27].

Seaweed-extract based biostimulants [28], particularly from the brown alga *Ascophyllum nodosum* (L.) Le Jolis [29,30], commonly used in the agriculture industry, have been observed to be beneficial for plants because of the cell signaling activity of some molecules, such as polysaccharides [24,31,32], polyphenols, peptides, carotenoids [33], betaines [34], and macro- and micronutrients. Moreover, phytohormones (e.g., auxins, gibberellins and cytokinins), found in seaweed extracts, accelerate metabolism and development [35,36], as well as other hormone-like substances [37–39]. Among the numerous commercial biostimulants, Goteo® (Goteo—Goactiv, UPL, Cesena, Italy), a biologically active filtrate called GA142, from the seaweed *A. nodosum*, is a source of polysaccharides, vitamins, auxins and cytokinins [40,41]. A previous study carried out by Loconsole et al. [42] proved that Goteo® improved the aerial and root quality traits of ornamental cuttings at a dose of 3 mL L^{-1} in wild sage, with a greater number of roots, better growth traits, root morphologies and carbohydrate content compared to IBA. Moreover, in glossy Abelia, the same authors [42] suggested the application of a 1 mL L^{-1} concentration of Goteo® to obtain high-quality rooted stem cuttings.

The biostimulant Kelpak® (Kelp Products Ltd., Cape Town, South Africa), an extract of brown seaweed, in particular *Ecklonia maxima* (Osbeck) Papenfuss, stimulates the growth and enlargement of the root system, due to the very high auxin-to-cytokinin ratio. This leads to a better absorption and translocation of macro- and microelements [43]. Moreover, the research carried out by Szabó et al. [44] on Prunus 'Marianna' showed that application of the Kelpak® biostimulant resulted in the highest rooting rate and increased the fresh weight of cuttings during rooting compared to the control. Finally, Traversari et al. [45] observed that the application of Kelpak® improved both rooting percentage and root biometric parameters on two rose cultivar cuttings, proving that a sustainable replacement of synthetic products used for rooting promotion is possible and desirable.

Red-tip photinia (*Photinia x fraseri* Dress.), a hybrid from *P. serrulata* Lindl. *x P. glabra* Thunb. Maxim., belonging to the *Rosaceae* family and discovered at the Fraser Nursery in Birmingham (AL, USA) around 1940, is an evergreen shrub. It is probably the most popular hedging plant of the last 20 years with great ornamental value; it has young red foliage and heads of long-lasting creamy flowers in late spring [46] and is widely used in the design of green areas [47]. Used by landscape designers as a low maintenance hedge or screen that provides spring interest, it attracts pollinating insects with its nectar

and the larvae of some species of *Lepidoptera* feed on its leaves. However, despite its important role in floriculture, this species is considered difficult to propagate [48]; although high concentrations of phytohormones are used to optimize rhizogenesis [49], limiting its commercial use [47]. *P. x Fraseri* 'Red Robin' differs from the species in that the young shoots are a particularly strong red color and it is particularly robust.

Despite many studies having illustrated the value of seaweed extracts in promoting growth, quality and yield when applied to the plant or rhizosphere in the production of cereals, fruit, vegetables and ornamental plants [50–52], their use in vegetative propagation by cutting has not been thoroughly investigated in terms of the current bibliography [53–56]. To our knowledge, there is currently no feasible enhanced method for vegetative propagation of *P. x fraseri* 'Red Robin' through stem cuttings by applying algae extracts as root promoters.

Given the economic importance of the species, this research aims to assess the effects of IBA and seaweed extract-based stimulators on the quality of photinia cuttings, in terms of rooting indicators and ground and aboveground agronomic features.

2. Materials and Methods

2.1. Rooting Environment

The experiment was conducted in a commercial greenhouse for propagation, located in Apulia, southern Italy (4054′19.1″ N, 1718′21.4″ E; 66 m a.s.l.), covered in an ethylene-vinyl acetate film with a net that provided 50% shading, from 4 March to 30 October 2021 (240 days after cutting, DAC). The greenhouse's environmental conditions during the experiment included: air temperatures that ranged from 12 °C at night to 27 °C during the day; seedbeds heated from the bottom during the winter; and misting for 60 s every 20 min (with droplets of an average size of 100 µm) from 8 a.m. to 6 p.m. during the summer period. The mist duration and interval varied in spring-autumn-winter months 2021.

2.2. Mother Plants and Cuttings

P. x fraseri 'Red Robin' stock mother plants were grown in open fields and regularly pruned to prevent flowering. Twenty mother plants were randomly selected. From each mother plant, ninety median and semi-hardwood stem cuttings were taken. Each cutting was selected for its uniformity, vigor, lack of disease, trueness to type, and a length of 4 cm with three nodes, removing the basal leaves and maintaining two leaves per cutting, with an average above-ground fresh mass of 0.73 g and an average total leaf area of 4.63 cm^2.

2.3. Rooting Promoters and Cutting Propagation

Cuttings were rooted using three different commercial rooting promoters: auxins were applied in the form of commercially available rooting powder: Rhizopon AA (identified as R), at 1% IBA (Sigma, St. Louis, MO, USA), directly to the bases of cuttings; the concentration was chosen according to previous studies that observed how high doses of IBA improve the quality of rooted cuttings. For example, refs. [57,58], have achieved the best results with 0.8%, while Bonamino and Blazich [49] have applied IBA at 1%. Kelpak® (identified as K), is composed of 34% (*w*/*w*) *E. maxima* extract 11.16 mg L^{-1} auxin and 0.031 mg L^{-1} cytokinin (with a auxin:cytokinin ratio of 360:1), alginates (1.5 L^{-1}), amino acids (total 441.3 mg 100 g^{-1}), mannitol (2261 mg L^{-1}), neutral sugars (1.08 g L^{-1}), and small amounts of macroelements (N 0.09%, P 90.7 mg kg^{-1}, K 7163.3 mg kg^{-1}, Ca 190.4 mg kg^{-1}, Mg 337.2 mg kg^{-1}, Na 1623.7 mg kg^{-1}) and microelements (mean composition: Mn 17.3 mg kg^{-1}, Fe 40.7 mg kg^{-1}, Cu 13.5 mg kg^{-1}, Zn 17.0 mg kg^{-1}, B 33.0 mg kg^{-1}) [43,59]. The (producer's) recommended dose is 10 mL L^{-1} before transplanting and the same dose three times after transplanting (7 days between each treatment); the dose chosen in our experiment was 2 and 3 mL L^{-1} respectively because they were applied by foliar application and not by drenching. Goteo® (Goteo—Goactiv, UPL, Italy) (identified as G) is a liquid formulation used as a source of auxins, cytokinins, polysaccharides and vitamins. GA142 is supplemented by the company with organic mineral fertilizers (*w*/*v*): 13% P_2O_5,

5% K_2O and 1.3–2.4% organic matter. In terms of concentration, the company recommends a 0.1% solution (1 mL L^{-1}) for vegetable crops, while ornamental plants are not specified. Previous experiments, from Gajc-Wolska [60] and Matysiak [61], suggested 3 to 4 treatments with 0.2% solution (2 mL L^{-1}) every 2 weeks to accelerate root regeneration.

On 4 March 2021, plastic trays with 100 holes and a diameter of 3.5 cm were sanitized using a fresh chlorine solution, which consisted of one part bleach (5.25 percent sodium hypochlorite) to nine parts water for a final strength of 0.5%. They were then stuffed with paper tubes that contained the substrate, a combination of brown and blonde peat and perlite (*v*:*v* = 80:20; pH 5.0–6.0; organic carbon, 35%; organic nitrogen, 0.8%; organic matter, 85%) (Jiffy® Products International BV, Toul, France). They were then saturated with water and cuttings were put two centimeters deep into the substrate.

2.4. Experimental Design

The experiment, started on 4 March, consisted of six treatments, each in three replications, each replication containing 100 cuttings.

The treatments applied were different concentrations of rooting stimulators compared to an untreated control:

- C0: distilled water;
- R1: Rhizopon AA, 1% IBA;
- K2: 2 mL L^{-1};
- K3: 3 mL L^{-1};
- G2: 2 mL L^{-1};
- G3: 3 mL L^{-1}.

IBA was applied directly to the bases of cuttings (10 mm) in the form of Rhizopon AA, the commercially available rooting powder. Before the application, the cutting base was wetted with distilled water.

Starting from 4 March, the Kelpak® and Goteo® solutions were sprayed with a hand sprayer every two weeks until they ran off the leaves of the cuttings, a total of 4 times. The treatment was always applied at the same time (9 a.m.) and the mist system stopped before the application, to prevent the solution being washed out by water.

The treatment design was a randomized complete block design of 18 experimental units (6 concentrations × 3 replicates).

Experimental analysis was conducted at the Floriculture and ornamental plants laboratory of the Department of Soil, Plant and Food Sciences, at the University of Bari.

2.5. Callus Initiation and Rooting Formation

At 70 DAC (13 May), callus initiation and rooting formation was investigated, sampling 24 cuttings per treatment (eight cuttings per replicate). The counting method was used to quantify the callus production rate and rooting percentage; gross morphological changes in the base of the stem cutting were captured using a Nikon SMZ800N microscope with a Nikon DS-Fi1 camera (Nikon Corporation, Tokyo, Japan) and Nis Elements 4.0 digital software at a resolution of 96 dpi. A cutting was considered rooted if it had at least one primary root ≥ 1 mm long; the unrooted cutting percentage was also evaluated.

On 13 May, 12 June (at 100 DAC) and 30 October (at 240 DAC), the callused (number of cuttings that produced calli/total number of cuttings × 100%) cuttings and rooted (number of rooted cuttings/total number of cuttings tested × 100%) cuttings percentages were recorded for twelve randomly chosen cuttings per treatment (four cuttings per replicate). At 240 DAC, the cutting survival percentage was also monitored. Percentage data were subjected to arcsine square root transformation before ANOVA analysis. At 240 DAC, the number of cuttings with 1, 2, 3, 4, 5 and 6 roots and the average root number per cutting for each treatment were recorded.

2.6. Roots Architecture

Twelve rooted cuttings from each treatment (four cuttings per replicate) at 240 DAC were sampled, and the morphology of the adventitious rooting system was examined. Each rooted cutting was cleaned, and the roots, leaves, and stems were separated. Water and a soft brush were used to gently wash out the rooting substrate. An Epson v700 Perfection (Japan) scanner was used to scan the roots at a resolution of 400 dpi. For the evaluation of total root length, root surface area, root diameter, number of root tips, forks, and crossings, the images were subsequently analyzed using image analysis software (WinRHIZO v. 2005b, Regent Instruments Inc., Québec, QC, Canada, www.regentinstruments.com accessed on 29 December 2022).

2.7. Ground Biomass

At 240 DAC, root fresh and dry weights (g) were measured on twelve rooted cuttings per treatment (four cuttings per replicate): fresh samples were dried in a thermo-ventilated oven at 70 °C until it reached a constant mass.

2.8. Aboveground Quality Features

At 240 DAC, the same samples analyzed for root parameters were recorded for aerial growth traits. Three new and fully opened leaves were sampled for analyzing the chlorophyll index (SPAD) (Konica Minolta Chlorophyll Meter SPAD-502 Plus, Solna, Sweden). The number of leaves per cutting was counted and the total leaf area per rooted cutting was measured with a leaf area meter (Delta-T; Decagon Devices, Pullman, WA, USA). Aboveground (leaves + stems) fresh and dry weights (g) were measured: samples were dried in a thermo-ventilated oven at 70 °C until it reached a constant mass.

2.9. Statistical Analysis

The effects of various rooting promoters doses on rooting performance and morphological features were examined using a one-way analysis of variance (ANOVA).

All the above data analyses were performed using SAS version 9.3 statistical software (SAS, 1999); treatment means were separated by the SNK (Student-Newman–Keuls) test ($p \leq 0.05$).

3. Results

Table 1 shows the percentage values of callus, rooted and unrooted cuttings at 70 DAC. A higher percentage of callused cuttings was observed with both seaweed extracts (K and G) at different concentrations compared to the IBA—Rhizopon treatment (R1) and the control. The percentage of callused cuttings was 84.3% on average in both the seaweed extracts, greater than 1.3 and 1.4 times compared to the control and the R treatment. The rooted cutting percentage was significantly higher in the R1 treatment (29.2%) followed by the G3 treatment (16.7%); the C0, K2, K3 and G2 treatments recorded the lowest rooted cutting percentage values, with no differences between them. The same table shows that the highest value of unrooted cuttings was found in the control (average 33.3%), while the cuttings treated with G3 all had calli or roots.

Figure 1 is a microscope image: we can see, on the left (a), the production of callous tissue in the G2 treated cutting; on the right (b), the adventitious root in the R1 treated cutting. Table 2 shows the callused and rooted cutting percentage at 100 and 240 DAC. At 100 DAC, the greatest callused cutting percentage values were obtained in the untreated control and Goteo® at the highest dose. The R1 application resulted in 51% fewer callused cuttings compared to the control; the same trend was found at 240 DAC. Furthermore, K3 and G3 showed a lower production of callused cuttings, compared to the corresponding lower dose of the same biostimulant (K3: 11.3%; G3: 10.3%; K2: 18.0%; G2: 15.3%). The same Table 2 shows that, at 100 and 240 DAC, the percentage of rooted cuttings treated under R1 was statistically the highest (25% at 100 DAC and 34.3% at 240 DAC), with an increase of 15.5% compared to the control at 240 DAC. At 100 DAC, treatments with

biostimulants showed the lowest values of rooting compared to IBA. At 240 DAC, the worst results were obtained by both biostimulant treatments at the highest doses, with no statistically significant differences between them (K3 = 21.3% and G3 = 20.7%).

Table 1. Callused, rooted and unrooted cutting (%) at 70 DAC in *P. x fraseri* 'Red Robin' influenced by rooting promoters concentration (RPC).

RPC	Cuttings (%)		
	Callused	**Rooted**	**Unrooted**
C0	66.7 ± 4.2 b	0.0 ± 0.0 c	33.3 ± 4.2 a
R1	58.3 ± 4.0 c	29.2 ± 3.0 a	12.5 ± 0.0 b
K2	87.5 ± 0.0 a	0.0 ± 0.0 c	12.5 ± 0.0 b
K3	83.3 ± 4.2 a	0.0 ± 0.0 c	16.7 ± 4.2 b
G2	83.3 ± 4.2 a	0.0± 0.0 c	16.7 ± 4.2 b
G3	83.3 ± 4.2 a	16.7 ± 4.2 b	0.0 ± 0.0 c

In columns, different letters indicate significant differences within parameters (S.N.K. test, $p \leq 0.05$; mean ± SD, $n = 3$) C0: untreated control; R1: Rhizopon AA (1% IBA); K: Kelpak®; G: Goteo®; K2: 2 mL L^{-1}; K3: 3 mL L^{-1}; G2: 2 mL L^{-1}; G3: 3 mL L^{-1}.

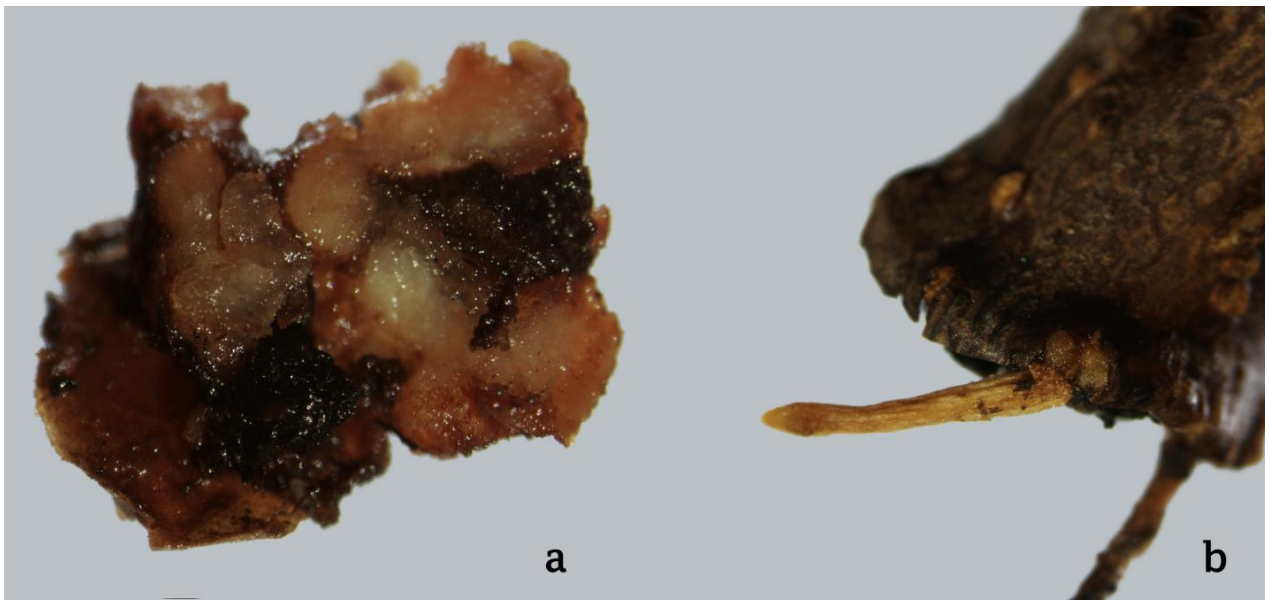

Figure 1. On the left (**a**) the production of callous tissue in G2 treated cutting; on the right (**b**) the adventitious root in R1 treated cutting.

Table 2. Callused and rooted cutting (%) at 100 and 240 DAC in *P. x fraseri* 'Red Robin' influenced by rooting promoters concentration (RPC).

RPC	Cuttings (%)			
	Callused		**Rooted**	
	100 DAC	**240 DAC**	**100 DAC**	**240 DAC**
C0	33.0 ± 1.1 a	13.7 ± 0.7 bc	7.7 ± 0.7 d	29.7 ± 1.4 b
R1	16.0 ± 1.0 d	3.3 ± 0.3 e	25.0 ± 1.0 a	34.3 ± 0.9 a
K2	30.0 ± 1.0 b	18.0 ± 1.1 a	12.0 ± 1.0 bc	25.0 ± 0.6 c
K3	29.0 ± 1.0 bc	11.3 ± 0.7 cd	13.3 ± 1.3 b	21.3 ± 0.7 d
G2	27.3 ± 0.9 c	15.3 ± 0.9 b	14.3 ± 1.3 b	27.0 ± 1.0 bc
G3	34.0 ± 0.6 a	10.3 ± 0.9 d	9.0 ± 1.6 cd	20.7 ± 0.9 d

In columns, different letters indicate significant differences within parameters (S.N.K. test, $p \leq 0.05$; mean ± SD, $n = 3$) C0: untreated control; R1: Rhizopon AA (1% IBA); K: Kelpak®; G: Goteo®; K2: 2 mL L^{-1}; K3: 3 mL L^{-1}; G2: 2 mL L^{-1}; G3: 3 mL L^{-1}.

Table 3 shows, at 240 DAC, the number of rooted cuttings per treatment with a different number of roots, between 1 and 6. Treatment with IBA positively influenced the number of cuttings with 3, 4, 5 and 6 roots.

Table 3. Cuttings (no.) with 1, 2, 3, 4, 5 and 6 roots at 240 DAC in *P. x fraseri* 'Red Robin' influenced by rooting promoters concentration (RPC).

RPC	Rooted Cuttings (No.)					
	1 Root	2 Roots	3 Roots	4 Roots	5 Roots	6 Roots
C0	13.7 ± 0.7 a	7.0 ± 0.6 a	4.3 ± 0.3 b	3.7 ± 0.3 a	1.0 ± 0.0 b	0.0 ± 0.0 c
R1	7.0 ± 0.6 c	7.0 ± 1.0 a	8.7 ± 0.3 a	4.3 ± 0.3 a	3.0 ± 0.6 a	4.3 ± 0.3 a
K2	10.3 ± 0.7 b	7.3 ± 0.3 a	4.3 ± 0.3 b	1.7 ± 0.3 b	1.0 ± 0.0 b	0.4 ± 0.3 bc
K3	9.7 ± 0.3 b	5.3 ± 0.3 a	2.7 ± 0.3 c	2.0 ± 0.0 b	1.0 ± 0.0 b	0.7 ± 0.3 bc
G2	14.0 ± 0.6 a	6.3 ± 0.3 a	2.0 ± 0.6 c	4.0 ± 0.6 a	0.7 ± 0.3 b	0.0 ± 0.0 c
G3	10.4 ± 0.7 b	5.7 ± 0.7 a	2.3 ± 0.3 c	1.3 ± 0.3 b	0.0 ± 0.0 b	1.3 ± 0.3 b

In columns, different letters indicate significant differences within parameters (S.N.K. test, $p \leq 0.05$; mean ± SD, $n = 3$) C0: untreated control; R1: Rhizopon AA (1% IBA); K: Kelpak®; G: Goteo®; K2: 2 mL L^{-1}; K3: 3 mL L^{-1}; G2: 2 mL L^{-1}; G3: 3 mL L^{-1}.

Data on root morphological features are provided in Tables 4 and 5. In Table 4, at 240 DAC, the statistically highest value of number of roots per cutting was obtained in the treatment carried out with the R1 application (3.07 roots per cutting). This was 50% higher than the average of all other treatments, which do not have statistically significant differences between them. The same table shows that the greatest root length development was obtained in cuttings treated with Rhizopon, compared to the other treatments; the applications of Kelpak® and Goteo® biostimulants at both concentrations resulted in an inhibition of root length with values below the untreated control. The same trend was recorded for the surface area.

Table 4. Root morphological traits: roots number (no.), length (mm), surface area (mm^2) and average diameter (mm), at 240 DAC in *P. x fraseri* influenced by rooting promoters concentration (RPC).

RPC	Roots (No.)	Length (mm)	Surface Area (mm^2)	Diameter (mm)
C0	2.0 ± 0.04 b	133.0 ± 1.1 b	23.8 ± 0.9 b	0.60 ± 0.01 a
R1	3.1 ± 0.07 a	162.1 ± 7.1 a	33.9 ± 0.9 a	0.73 ± 0.02 a
K2	2.1 ± 0.10 b	54.9 ± 3.0 cd	12.5 ± 0.4 c	0.67 ± 0.02 a
K3	2.1 ± 0.05 b	51.1 ± 2.3 d	11.8 ± 0.6 c	0.66 ± 0.01 a
G2	1.9 ± 0.08 b	67.6 ± 4.0 cd	14.5 ± 0.1 c	0.70 ± 0.02 a
G3	2.0 ± 0.07 b	72.2 ± 7.0 c	16.3 ± 0.2 c	0.73 ± 0.01 a

In columns, different letters indicate significant differences within parameters (S.N.K. test, $p \leq 0.05$; mean ± SD, $n = 3$) C0: untreated control; R1: Rhizopon AA (1% IBA); K: Kelpak®; G: Goteo®; K2: 2 mL L^{-1}; K3: 3 mL L^{-1}; G2: 2 mL L^{-1}; G3: 3 mL L^{-1}.

Table 5. Root morphological traits: root tips (no.), forks (no.) and crossing (no.), at 240 DAC in *P. x fraseri* influenced by rooting promoters concentration (RPC).

RPC	Root		
	Tips (No.)	Forks (No.)	Crossings (No.)
C0	330 ± 7.0 b	755 ± 12.5 b	99 ± 1.8 b
R1	677 ± 5.6 a	1215 ± 7.7 a	114 ± 3.8 a
K2	217 ± 1.1 c	326 ± 6.3 e	25 ± 1.1 e
K3	225 ± 6.7 c	298 ± 5.5 e	30 ± 1.5 e
G2	226 ± 3.8 c	459 ± 7.9 d	38 ± 0.6 d
G3	340 ± 8.7 b	621 ± 13.3 c	49 ± 1.7 c

In columns, different letters indicate significant differences within parameters (S.N.K. test, $p \leq 0.05$; mean ± SD, $n = 3$) C0: untreated control; R1: Rhizopon AA (1% IBA); K: Kelpak®; G: Goteo®; K2: 2 mL L^{-1}; K3: 3 mL L^{-1}; G2: 2 mL L^{-1}; G3: 3 mL L^{-1}.

Table 5 shows the average number of tips, forks and crossings at 240 DAC. Cuttings treated with R1 showed a 105% increase in tips compared to the untreated control and G3. The K2, K3 and G2 treatments were equal to each other and below the control, by an average of 32%. Regarding the number of forks and crossings (Table 5), the highest value was obtained with cuttings treated with Rhizopon, +61% forks and +15% crossings compared to the control; the cuttings treated with the biostimulants at the different concentrations obtained lower values than the control.

Data on fresh and dry ground weights are provided in Table 6: rooted cuttings under R1 show the highest, and statistically different, dry value (0.35 g) compared to the other treatments, resulting in +40% in comparison to the control.

Table 6. Ground fresh and dry weights (g) per cutting, at 240 DAC in *P. x fraseri* influenced by rooting promoters concentration (RPC).

RPC	Ground Weights (g)	
	Fresh	Dry
C0	0.81 ± 0.07 ab	0.25 ± 0.09 b
R1	0.97 ± 0.02 a	0.35 ± 0.01 a
K2	0.47 ± 0.02 c	0.15 ± 0.01 c
K3	0.72 ± 0.02 b	0.23 ± 0.01 b
G2	0.81 ± 0.07 ab	0.24 ± 0.01 b
G3	0.65 ± 0.03 b	0.18 ± 0.01 c

In columns, different letters indicate significant differences within parameters (S.N.K. test, $p \leq 0.05$; mean ± SD, $n = 3$) C0: untreated control; R1: Rhizopon AA (1% IBA); K: Kelpak®; G: Goteo®; K2: 2 mL L^{-1}; K3: 3 mL L^{-1}; G2: 2 mL L^{-1}; G3: 3 mL L^{-1}.

Table 7 shows the aboveground morpho-biometric features: no statistically significant differences were found between the treatments, in terms of the number of leaves per cutting and chlorophyll index. At 240 DAC, the statistically highest value of leaf area per cutting was obtained in the treatment carried out with the R1 application (27.9 cm^2); K3 and G3 were similar to the control, with lower values compared to R1. The worst performances were observed in the K2 and G2 treatments.

Table 7. Above-ground morpho-biometric traits: leaves per cutting (no.), leaf area (mm^2) and chlorophyll index (SPAD), at 240 DAC in *P. x fraseri* influenced by rooting promoters concentration (RPC).

RPC	Leaves per Cutting (No.)	Chlorophyll Index (SPAD)	Leaf Area per Cutting (cm^2)
C0	3.7 ± 0.7 a	570 ± 13.7 a	22.0 ± 1.2 b
R1	4.7 ± 0.3 a	530 ± 11.9 a	27.9 ± 0.8 a
K2	3.3 ± 0.3 a	533 ± 15.0 a	18.1 ± 0.3 c
K3	4.0 ± 0.6 a	554 ± 2.91 a	21.9 ± 0.9 b
G2	3.3 ± 0.3 a	529 ± 20.4 a	18.4 ± 0.4 c
G3	4.0 ± 0.6 a	582 ± 11.6 a	22.3 ± 1.4 b

In columns, different letters indicate significant differences within parameters (S.N.K. test, $p \leq 0.05$; mean ± SD, $n = 3$) C0: untreated control; R1: Rhizopon AA (1% IBA); K: Kelpak®; G: Goteo®; K2: 2 mL L^{-1}; K3: 3 mL L^{-1}; G2: 2 mL L^{-1}; G3: 3 mL L^{-1}.

Table 8 showed that the aboveground dry weight was significantly influenced by treatments: the rooting effect under IBA (R1) was higher than that under the control and K3.

Table 8. Aboveground fresh and dry weights (g) per cutting, at 240 DAC in *P. x fraseri* influenced by rooting promoters concentration (RPC).

RPC	Above-Ground Weights (g)	
	Fresh	Dry
C0	1.11 ± 0.14 a	0.40 ± 0.09 b
R1	1.29 ± 0.13 a	0.47 ± 0.11 a
K2	0.99 ± 0.11 a	0.35 ± 0.04 bc
K3	1.11 ± 0.09 a	0.39 ± 0.08 b
G2	0.96 ± 0.08 a	0.33 ± 0.08 c
G3	1.04 ± 0.06 a	0.35 ± 0.03 bc

In columns, different letters indicate significant differences within parameters (S.N.K. test, $p \leq 0.05$; mean ± SD, $n = 3$) C0: untreated control; R1: Rhizopon AA (1% IBA); K: Kelpak®; G: Goteo®; K2: 2 mL L^{-1}; K3: 3 mL L^{-1}; G2: 2 mL L^{-1}; G3: 3 mL L^{-1}.

4. Discussion

Calkins [62] reported that Fraser's photinia is an important primary ornamental landscaping species with optimal hedge or screen effects and low maintenance, but it is difficult to root when propagated by cuttings. Given the economic importance of the species in the ornamental industry, this research aims to assess the effects of IBA and algae extract-based stimulators on the quality of photinia cuttings, in terms of rooting indicators and ground and aboveground agronomic features. Vegetative propagation by cuttings has numerous advantages, one of which is to provide more uniform and agronomically superior commercial plantlets than those obtained by heterozygous seeds [63]. First results show different responses to adventitious rhizogenesis under in-dole-3-butyric acid and seaweed extract treatments. The application of exogenous auxin and cytokinin stimulates callus differentiation in various species [64]. Several studies showed that an intermediate ratio of auxin and cytokinin promotes callus induction, while a high ratio of auxin-to-cytokinin or cytokinin-to-auxin induces root and shoot regeneration, respectively [65]. Plants of *Arabidopsis thaliana* treated with a biostimulant extracted from *A. nodosum* showed an increase in cytokinin-like responses, suggesting a contribution in the cytokinin-like activity of the extracts's compounds [66]. From our results obtained at 70 DAC, it would appear that the seaweed extracts stimulated the production of over 80% of cuttings with callus (Figure 1, Table 1). Comparing this result to IBA (R1), it can be observed that this, in contrast, obtained the highest rooting percentage (Table 1). Furthermore, it would appear that the conspicuous production of callus tissue prevents the adventitious rooting of photinia cuttings, representing a structural obstacle to the emission of adventitious roots. Our preliminary results agree with Monder et al. [67]: in the rhizogenesis of the 'Hurdal' rose, the authors showed that an increase in rooting percentage was only strictly connected to a decrease in the percentage of cuttings with calli only. Callus overgrowth in photinia could be an unfavorable phenomenon for fast rhizogenesis. Conversely, Costa et al. [68] in *Rosa* 'Madelon' and Fouda and Schmidt [69] in *Rosa rugosa* stem cuttings found that the new parenchyma tissue (callus) precedes root initiation. Martins et al. [70] showed an inverse relationship between rooting and callus formation for olive tree cuttings. In our study, the application of IBA (R1) improved the rooting percentage at 240 DAC (Table 2) and the number of cuttings with six roots (Table 3), as compared to the untreated control and seaweed extracts. In our experimental conditions, the application of IBA at the concentration of 1 gL^{-1} produced 33% of rooted cuttings, while Bonaminio and Blazich, [49] in 1983, found that 5000 and 10,000 mg L^{-1} of IBA solutions applied to the terminal, semi-hardwood cuttings of Fraser's photinia, promoted rooting more effectively than the control, and increased rooting percentage significantly (100 and 93%). Cutting success, entailing quality AR formation with high rooting percentage, depends on numerous factors, such as cutting type, environmental conditions, nutritional levels of the stock plant, rooting medium and phytohormone application [17,71,72]. IBA has been reported to increase in vivo adventitious root formation, overall quality and uniformity of roots in many ornamental species [73–82]. Untreated stem

cuttings (C0) were also able to root, but with a lower rooting percentage, in comparison to IBA-treated cuttings, possibly due to the presence of stored carbohydrates and endogenous auxin contents in the cuttings [83]. Until now, no research has compared the morphological quality of adventitious roots treated with different IBA and seaweed extract concentrations in Fraser's Photinia cuttings. To improve plant performance and provide protection against the deleterious effects of numerous abiotic stressors, several amendments such as biostimulators and bioelicitors have been used [84]. The favourable impact of using seaweed extracts as natural regulators has resulted in better crop growth and production [85,86]. In our experimental conditions, neither K nor G biostimulants, at different concentrations, increased the rooting percentage (Table 2) and the root architecture (Figure 2, Tables 4 and 5) compared to the IBA treatment. On the contrary, previous studies have shown the efficacy of biostimulants in promoting rhizogenesis. For example, the use of a 40% concentrated *A. nodosum* extract increased the rooting of *Passiflora actinia* by about 10% [87]. Even if unsuccessful, our results in the treatments with biostimulants agree with those of Traversari et al. [45] on rose rhyzogenesis: cuttings treated with Phylgreen, a commercial biostimulant made from *A. nodosum*, through a low temperature mechanical extraction, had low values of both survival rate and root biometric parameters. Since biostimulating effects are clearly species-specific and product-specific, results regarding one biostimulant or one species only do not directly apply to another biostimulant or another plant species [51,88,89]. In our study, both Kelpak® and Goteo® negatively affected root length, surface area and diameter compared to IBA and the control (Table 4). On the contrary, positive effects of Goteo® on rooting were reported for *Physocarpus opulifolius* [75], *Hydrangea paniculata* [90], *Ornithogalum arabicum* [91] and rose [58]. In Fraser's Photinia, both Kelpak® and Goteo® at different concentrations decreased the number of root tips, forks and crossings compared to the IBA treatment (Table 5). Based on these findings, the overall development and morphology of Photinia cuttings treated with Kelpak® and Goteo® were inhibited.

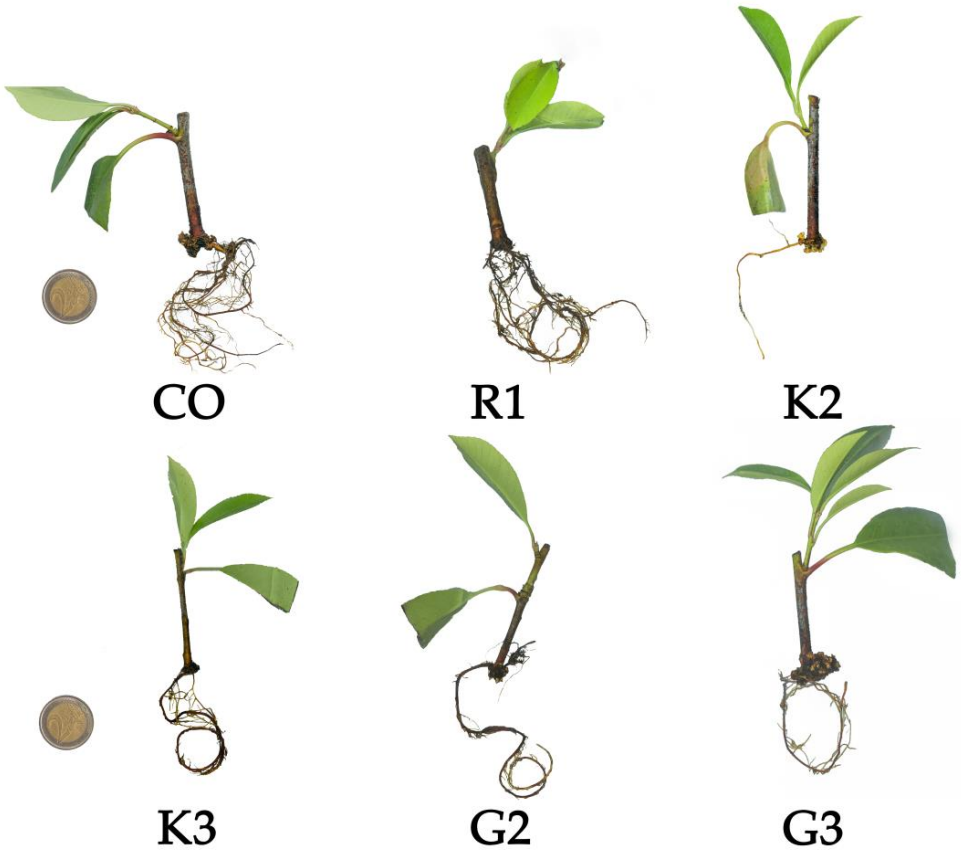

Figure 2. Root architecture in *P. x fraseri* 'Red Robin' influenced by application of rooting promoters concentration.

Seaweed extracts often stimulate and accelerate cell division, elongation, differentiation and protein synthesis [37,92]; Makhaye et al. [93] have verified the potential stimulatory effect of biostimulants (especially Kelpak®) on the germination of *A. esculentus* seeds. The application of biostimulants based on seaweed extracts in our experiment did not positively influence the number of leaves, chlorophyll index (SPAD) and leaf area index (Table 7). These results are in disagreement with that found in *Lantana camara*, *Abelia x grandiflora* [42] and *Cornus alba* 'Aurea' and 'Elegantissima' [94]. Our preliminary results (Tables 6 and 8) disagree with Ratore et al. [95], Kocira et al. [96], Gajc-Wolska et al. [60] and Caccialupi et al. [97] who obtained a positive effect, by applying seaweed extracts, on plant growth, development and yield. Our results agree with Francke et al. [41], who exhibited a lower yield of shallots than that of the control (4%) by applying Goteo biostimulant. The limited information available in the literature does not allow further discussion of this biostimulant since contrasting results were observed in Fraser's photinia 'Red Robin' cuttings with respect to other investigated species. The overall quality of cuttings in the IBA treatment was the strongest, with 1000 mg L^{-1} being the optimum concentration, according to studies by Quan et. al. [98].

5. Conclusions

In conclusion, the main outcomes of this study can be summarized as follows: we suggest that the use of 1% IBA (Rhizopon AA), compared to an untreated control, may be beneficial to ornamental nursery farmers wishing to produce Fraser's photinia 'Red Robin' quality cuttings with a well-developed root system and, therefore, capable of achieving rapid establishment at transplantation. On the contrary, neither seaweed extract, Kelpak® or Goteo®, at different concentrations, improved root percentage and architecture, and ground and above ground weights of rooted cutting, compared to the untreated control. This result indicates that these natural products are not suitable for Fraser's photinia 'Red Robin' propagation using this methodology. Further research must be conducted to propose effective agronomic protocols by investigating application methods, doses and number of applications, and to clarify the biochemical and molecular mechanisms of action of these seaweed extracts.

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