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# Application of commercial seaweed extract-based biostimulants to enhance adventitious root formation in ornamental cutting propagation protocols: a review

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Despite significant advancements in stem-cutting propagation, insufficient rooting efficiency remains an economic burden for the ornamental nursery industry. IBA and NAA play a critical role in generating adventitious roots (AR) when applied exogenously. In sustainable agriculture, the substitution of chemical inputs, with alternative natural eco-friendly products presents a key challenge. Biostimulants can form part of a solution to mitigate such risks deriving from the use of agrochemicals, they are generally considered to be non-toxic, non-polluting, biodegradable, and non-hazardous. The current knowledge of the use of commercial seaweed extract (SE) products applied to ornamental cutting propagation has not been summarized until now. Therefore, we conducted a systematic review, and we hypothesized that SE-based biostimulant application to ornamental stem cuttings improves AR formation in terms of rooting percentage, root number, and architecture. Moreover, they increase the overall quality of a rooted cutting as dry biomass and organic compound content. The authors chose SE-based biostimulants because they have been proven to have an extremely low carbon footprint; moreover, they are expected to account for more than 33% of the global market for biostimulants and reached a value of 894 million Euros by 2022. This review focuses on (i) SE-based biostimulants, in particular, brown algae; (ii) technical information on five commercial products: Goteo<sup>®</sup>, Kelpak<sup>®</sup>, AlgaminoPlant, Bio Rhizotonic, Actiwawe and others, less known, also used as phyto regulators substitutes; (iii) applied protocols, describing dose, application method, number of treatments, cutting type; (iv) effects of applied protocols on rooting rate, root architecture and overall rooted cutting quality. Outcomes show that findings vary based on crops, cuttings, location, raw materials, composition, dose, application number and procedures, and growth environment.

## KEYWORDS

AlgaminoPlant, Bio Rhizotonic, Goteo<sup>®</sup>, Kelpak<sup>®</sup>, phyto regulator, root architecture, rooting percentage

## 1 Introduction to ornamental stem cutting issues

Plant propagation is a vital part of both the agricultural and horticultural industries. Ornamentals such as rose, glossy abelia, wild sage, red tip photinia, ninebark, Pennisetum and others can be propagated using either vegetative multiplication, which produces genetically identical clones, or sexual reproduction (Hartmann and Kester, 2002; Winkelmann, 2013). For the industrial ornamental nursery, seed germination can be unreliable due to dormancy (Maghdouri et al., 2021) and seeds often have low viability (Cafourek et al., 2021). Vegetative propagation is insisted by farmers due to its lower cost, ease, and speed compared to sexual reproduction. Moreover, the resulting plants are clones that maintain the same morpho-physiological and genetic characteristics as the parent plants, ensuring uniformity and early production (Kentelky et al., 2021; Tsaktsira et al., 2021). Various techniques can be used for the ornamentals vegetative propagation: while layering is a simple technique, it is costly and only produces a limited number of clones (Braun and Wyse, 2019), grafting allows for adaptation to unfavorable environmental conditions and resistance to soil-borne pathogens and parasites but is labor-intensive and can lead to compatibility issues between the rootstock and graft (Baron et al., 2019; Rasool et al., 2020; Lesmes-Vesga et al., 2021). 'In vitro' clonal propagation can produce many plants in a short time (Gianguzzi et al., 2020; Park et al., 2021) but requires specialized laboratory facilities and skilled labor (Read, 2011).

Propagation by stem cutting, less expensive and easier than the 'in vitro' technique, is the method most widely used to multiply clones of landscape woody (Kaviani and Negahdar, 2017; Druege et al., 2019; Ross et al., 2021) and herbaceous ornamentals. Adventitious roots (AR) "arise from non-root parts of plants, in response to stress and wounding" (Gogna et al., 2022) and are a key step in stem-cutting propagation (Assis et al., 2004; Oinam et al., 2011) of economically important landscaping ornamentals (Geiss et al., 2009).

The evaluation of the rooting quality of propagated cuttings is highly relevant from an economical viewpoint. AR quality is characterized by a high both percentage of rooted cuttings and the number and length of very fine roots, which are essential for continuous access to water and nutrients (Atkinson, 2000) and can help the plant withstand transplant shock, increasing survival and plant growth (Franco et al., 2011; Khan et al., 2016; Koevoets et al., 2016) and by an adequate number of higher caliber roots with mechanical support function. Indeed, inadequate environmental, nutritional and hormonal conditions of stem cutting can lead to the production of insufficient AR, with consequent loss of quality. The overall cutting quality is represented too by dry biomass of above-ground and ground parts and by some organic compound (chlorophyll and carbohydrate) content.

During the last few decades, intense interest has emerged in the role of other potential rooting co-factors, such as carbohydrates, phenolics, polyamines, and other plant growth regulators, as well as signal molecules, such as reactive oxygen and nitrogen species (Druege, 2023; Roussos, 2023).

The technical innovations for propagation by cuttings (automatic mist propagation unit, basal heating) provide optimal environmental conditions to improve rooting efficiency. Regarding stock plants nutritional conditions, it is widely recognized that, having adequate carbohydrate reserves, specifically soluble sugars, is crucial for survival and AR formation, particularly under unfavorable environmental conditions for photosynthesis (Hartmann and Kester, 2002). This is particularly relevant to producing young ornamental plants in Central Europe, where cuttings are rooted at relatively low irradiance levels, while in East and Central Africa and Latin America they are rooted under high irradiance conditions. This is because cuttings are unable to reach their light compensation point under low light conditions, especially during winter (Ahkami et al., 2009; Druege, 2009; Lohr et al., 2017; Tahir et al., 2022). A significant portion of sucrose is converted into starch, which is believed to be the primary carbon source for the growth of AR (Lohr et al., 2017).

Auxin is one of the major endogenous hormones involved in the process of adventitious rooting and the physiological stages of rooting are correlated with changes in endogenous auxin concentrations (Justamante et al., 2019). Indole-3-acetic acid (IAA) is the most abundant member of the auxin family of phytohormones, and its biosynthesis in plants and bacteria proceeds through tryptophan-dependent and independent pathways; it plays an important role in cell division and root initiation (Luziatelli et al., 2020).

Generally, on farms, the application of a specific synthetic plant phytohormone (i.e. auxin) can enhance the rooting percentage, number and quality of AR and shorten the rooting time in some species but with little effect on others (Díaz-Sala, 2021; Justamante et al., 2019).

Compounds such as Indole-3-butyric acid (IBA) and 1-Naphthaleneacetic acid (NAA) play a critical role in generating AR when applied exogenously to cuttings (Hac-Wydro and Flasiński, 2015; Gonin et al., 2019). The IBA-containing preparation of Rhizopon and the water IBA solution positively affected the degree of rooting and the percentage of rooted cuttings in deciduous leafy shrubs (Pacholczak et al., 2016a). Loconsole et al. (2022a) experimented to improve the cutting propagation protocol and rooting quality of wild sage (*Lantana camara*) and glossy abelia (*Abelia x grandiflora*) treated with 0, 1,250, 2,500, and 5,000 mg L<sup>-1</sup>. Results showed that the species had remarkable sensitivity to IBA dosage, mostly for AR quality, reaching the best results with 5,000 mg L<sup>-1</sup> IBA in *L. camara* 'Yellow' (increased root number, total length, surface area and the number of forks and crossings), and 1,250 mg L<sup>-1</sup> *A. x grandiflora* 'Edouard Goucher'.

More than 30 commercial products containing IBA have been registered by the United States Environmental Protection Agency for utilization on fruit, vegetable, and ornamental crops, which are categorized as biochemical pesticides (United States Environmental Protection Agency, 2022). IBA hazardous information (Globally Harmonized System of Classification and Labelling of Chemicals GHS Classification, US), directed to operators in the nursery sector, is represented by acute toxicity (Category 3), skin corrosion

(Category 2), eye irritation (Category 2A), acute aquatic toxicity (Category 3), chronic aquatic toxicity (Category 3).

## 2 Biostimulants in sustainable agriculture

In sustainable agriculture, the substitution of chemical inputs, such as growth regulators too, with alternative natural eco-friendly products presents a key challenge (Bulgari et al., 2020; Malik et al., 2021; Carillo et al., 2022). Biostimulants could form part of a solution to mitigate such risks deriving from the use of agrochemicals (Lucini et al., 2020; Loconsole et al., 2022b). Several concepts have been proposed to define plant biostimulants, referred to as eco-friendly because they are generally considered to be non-toxic, non-polluting, biodegradable, and non-hazardous (Yakhin et al., 2017). Biostimulants are either organic, inorganic, or microbial substances, that can enhance in small amounts, the growth, yield and quality of crops (Rouphael and Colla, 2018; Sible et al., 2021; Kisvarga et al., 2022; Wei et al., 2022; Ganugi et al., 2023; Kaushal et al., 2023; Rouphael et al., 2023). They also provide anti-stress effects (Ambrosini et al., 2021; González-Morales et al., 2021; Franzoni et al., 2022; Jacomassi et al., 2022; Ma et al., 2022; El-Nakhel et al., 2023; Zhang et al., 2023). Li et al. (2022) reported a comprehensive meta-analysis on non-microbial biostimulants of over one thousand pairs of open-field data in a total of 180 qualified studies worldwide. They are frequently used in horticulture (Baltazar et al., 2021) but rarely in plant propagation. In 2014, Calvo defined biostimulants as any substances or microorganisms that benefit the plant (Calvo et al., 2014), while Du Jardin et al. stated, in 2015, that the definition is based on what they are not, rather than what they are; for instance, fertilizers and pesticides increase plant yields but do not fall into the biostimulants category. Most biostimulants are complex mixtures and on this basis (Povero et al., 2016), two definitions have been given and both share the requirement that the mode of action must be unknown but differ in the fundamental assumption that the function of the biostimulant is a consequence of the discrete components (Carletti et al., 2021; Pandey et al., 2022) or a consequence of the 'emergent' properties of the biostimulant. Therefore, Yakhin et al. (2017) have developed a third concept that integrates the two previous concepts 'a formulated product of biological origin that improves plant productivity as a consequence of the novel, or emergent properties of the complex of constituents, and not as a sole consequence of the presence of known essential plant nutrients, plant growth regulators, or plant protective compound'.

In this review, the authors classified biostimulants into eight groups: (1) PGPR beneficial bacteria, (2) AMF beneficial fungi, (3) SE Seaweed extracts; (4) Ple Plant extracts, (5) PH protein hydrolysates and other nitrogen-containing compounds, (6) HFA humic acid and fulvic acid, (7) Ch chitosan and other biopolymers, and (8) Si silicon, having as references papers of du Jardin (2015) and Yakhin et al. (2017).

Actually, "Fertilising Products Regulation (2019/1009)" represents a huge step forward for plant biostimulants (European Commission, 2019): for the first time, EU law recognizes biostimulants, and there is a

common definition across all the Member States. The agricultural sector has been challenged to increase productivity to feed the growing global population while reducing adverse impacts on ecosystems and human health (Rouphael and Colla, 2020). One solution to reduce chemical use, without compromising crop quality and production (Li et al., 2022), could be by using biostimulants (Zulfiqar et al., 2020), even if their effects and mechanisms of action are still not fully understood (Toscano et al., 2018). The recent Green Deal Europe guidelines will probably increase the research and innovation in the biostimulants sector (Corsi et al., 2022).

Despite significant advancements in stem-cutting propagation, insufficient rooting efficiency remains an economic burden for the horticultural nursery industry (Ahkami et al., 2009). The current knowledge of the use of commercial SE products applied to ornamental cutting propagation has not been summarized until now.

Therefore, we conducted a systematic review to update evidence presented in scientific articles from 2011 to 2023: we hypothesize that commercial SE-based biostimulant products application to ornamental stem cuttings improves AR formation in terms of rooting percentage, root number, and overall quality of rooted cutting.

The authors chose SE-based biostimulants because they have been proven to have an extremely low carbon footprint (Ghosh et al., 2015); moreover, they are expected to account for more than 33% of the global market for biostimulants and reached a value of 894 million Euros by 2022 (El Boukhari et al., 2020).

The treated areas in this review could fill the gaps in knowledge on the SE applications in clonal propagation by cutting. It is undoubtedly thanks to the reviews of Kisvarga et al. (2022) on the use of biostimulants in ornamental greenhouse production (i.e. micro propagated orchids, endangered Brazilian, gladioli and seed-sown species) and Parađiković et al. (2019) on pot wild roses, marigold, and zinnia that ornamental crops have the place they deserve in the general landscape of biostimulants in horticulture.

## 3 Methodology

This review focuses on ornamental commercially important crops (Table 1). Demand for ornamentals has been increasing lately, due to the dynamic development of urban infrastructure (Francini et al., 2022). Green areas have become an inseparable element of modern settings. In response to this trend, ornamental nursery production has been increasing to the point of becoming the most profitable branch of ornamental horticulture.

The applied methodology, shown in Figure 1, was divided into five successive phases:

1. *Formulation of the problem*: Despite significant advancements in stem-cutting propagation, insufficient rooting efficiency remains an economic burden for the floricultural nursery industry.
2. *Objectives*: to describe: (i) SE-based biostimulants; (ii) technical information on five commercial SE-based

**TABLE 1** List of references regarding ornamental commercially important crops propagated by cutting using SE-based biostimulants with (+) or without (-) phyto regulators (PhR). (n=21).

Scientific Name	Common Name	SE(*)/PhR(**)	Reference (n=21)
<i>Photinia x fraseri</i> 'Red Robin'	Red tip photinia	SE+PhR	Loconsole et al. (2023)
Rose 'Duchesse d'Angoulême', 'Hurdals', 'Maiden's Blush' 'Mousseuse Rouge', <i>R. beggeriana</i> 'Polstjärnan', <i>R. helenae</i> 'Semiplena'	Rose	SE+PhR	Monder and Pacholczak (2023)
<i>Abelia x grandiflora</i> and <i>Lantana camara</i>	Glossy abelia and Wild sage	SE+PhR	Loconsole et al. (2022b)
<i>Crassula ovata</i>	Jade plant	SE-PhR	Toża et al. (2022)
R. 'Cosmos'® and 'Michelangelo'®	Rose	SE+PhR	Traversari et al. (2022)
R. 'Hurdal'	Rose	SE+PhR	Monder et al. (2021)
<i>Conocarpus erectus</i>	Conocarpus	SE+PhR	Abdel-Rahman et al. (2020)
<i>Pennisetum</i> 'Vertigo'	Pennisetum	SE-PhR	Kapczynska et al. (2020)
R. 'Duchesse d'Angoulême'	Rose	SE+PhR	Monder and Pacholczak (2020)
R. 'Elfrid' and 'Weisse Immensee'	Rose	SE+PhR	Pacholczak and Nowakowska (2020)
<i>Passiflora actinia</i>	Passiflora	SE-PhR	Gomes et al. (2018)
R.'Hurdals', 'Maiden's Blush', 'Mousseuse Rouge', <i>beggeriana</i> 'Polstjärnan', and <i>helenae</i> 'Semiplena'	Rose	SE+PhR	Monder and Pacholczak (2019)
<i>Cornus alba</i>	Dogwood	SE+PhR	Pacholczak et al. (2017)
<i>Physocarpus opulifolius</i>	Ninebark	SE+PhR	Pacholczak et al. (2016b)
<i>Physocarpus opulifolius</i>	Ninebark	SE+PhR	Pacholczak et al. (2016a)
<i>Cotinus coggygria</i>	Smoke tree	SE+PhR	Pacholczak et al. (2015)
R. 'Duchesse d'Angoulême'	Rose	SE+PhR	Monder et al. (2014)
<i>Ornithogalum umbellatum</i>	Star of Bethlehem	SE-PhR	Salachna et al. (2014)
<i>Camellia japonica</i> L.	Common camelia	SE-PhR	Ferrante et al. (2012)
<i>Pelargonium peltatum</i> 'Ville de Paris Red'	Pelargonium	SE-PhR	Krajnc et al. (2012)
<i>Prunus 'Marianna'</i>	Marianna plum	SE-PhR	Szabó et al. (2011)

SE(\*): Seaweed extract; PhR(\*\*): Phyto regulators

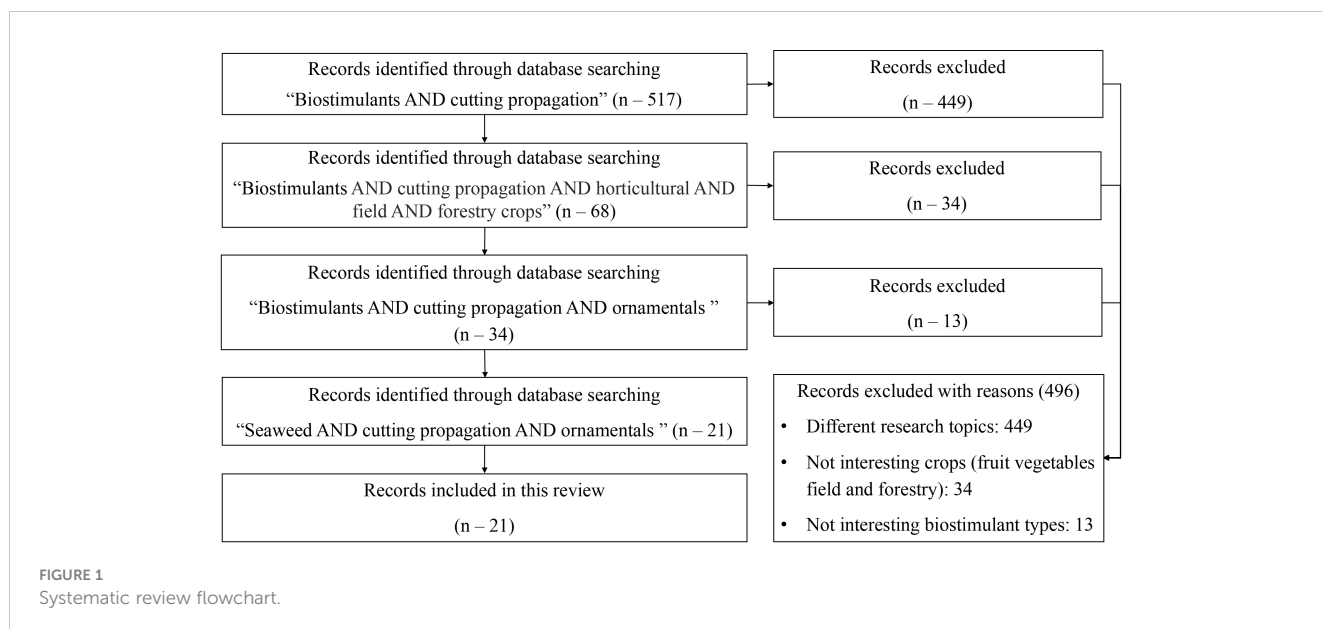
products: Goteo®, Kelpak®, AlgaminoPlant, Bio Rhizotonic, Actiwawe and some others less known also used as phyto regulators substitutes; (iii) applied protocols: crop, cutting type, commercial SE product, dose, application method, number of treatments; (iv) effects of applied protocols on rooting rate, root architecture and overall quality.

3. **Keyword identification:** Thomson Reuters' Web of Science, Elsevier's Scopus, and Google Scholar were queried until November 2023 for published scientific publications, written in English and identified using the following keywords: in the first step, "biostimulants AND cutting propagation AND horticulture AND field AND forestry crops" (n=68); in the second: "biostimulants AND cutting propagation AND ornamentals" (n=34), in the third: "Seaweed extract biostimulants AND cutting propagation AND ornamentals" (n=21) through the process of identification of bibliographical references and discarding those irrelevant.
4. **Evaluation by title/keywords/abstract:** n=21 (Supplementary Table 1).
5. **Critical reading and text analysis of papers**

The processing of bibliographic data shows that ornamental crops constitute as much as 45% of all those propagated by stem cuttings applying biostimulants (Figure 2). Figure 3 focuses on the comparison between the different types of biostimulants mentioned in the cutting propagation protocols; SE has higher relative frequency values both in horticultural (field, forest, fruit, vegetable and ornamental crops) and ornamental crops.

## 4 SE-based biostimulants

Seaweeds are a diverse group of organisms, with approximately 9,000 species of red, brown, and green seaweeds identified. Among these groups, brown seaweeds (*Phaeophyta*) are commonly used for commercial extract manufacturing in agriculture (Atzmon and van Staden, 1994) and horticulture (Khan et al., 2009), due to their high content of macro- and micronutrients, amino acids, polyunsaturated fatty acids, vitamins, and polysaccharides such as alginates, fucoidans, laminarians, lichenan-like glucans, and fucose containing glucans (Khan et al., 2009; Battacharyya et al., 2015; Shukla et al., 2016). The biostimulant function of SE is commonly associated with the content of phytohormone, such as cytokinins, auxin, gibberellin and other hormone like compounds (Petropoulos, 2020; Stirk et al., 2020; Ali et al., 2021; Cardarelli et al., 2024). These compounds can act directly (i.e. exogenous apport) or indirectly [i.e. stimulation of genes expression and promoting the endogenous biosynthesis of auxin, cytokinins and gibberellins (Ali et al., 2021)], stimulating plant growth and development (Jannin et al., 2013; Stirk and Van Staden, 2014; Elansary et al., 2017) and improving nutrient uptake (González et al., 2013). All these components have a synergistic effect in the crops, providing a strong root system, promoting plant growth, and

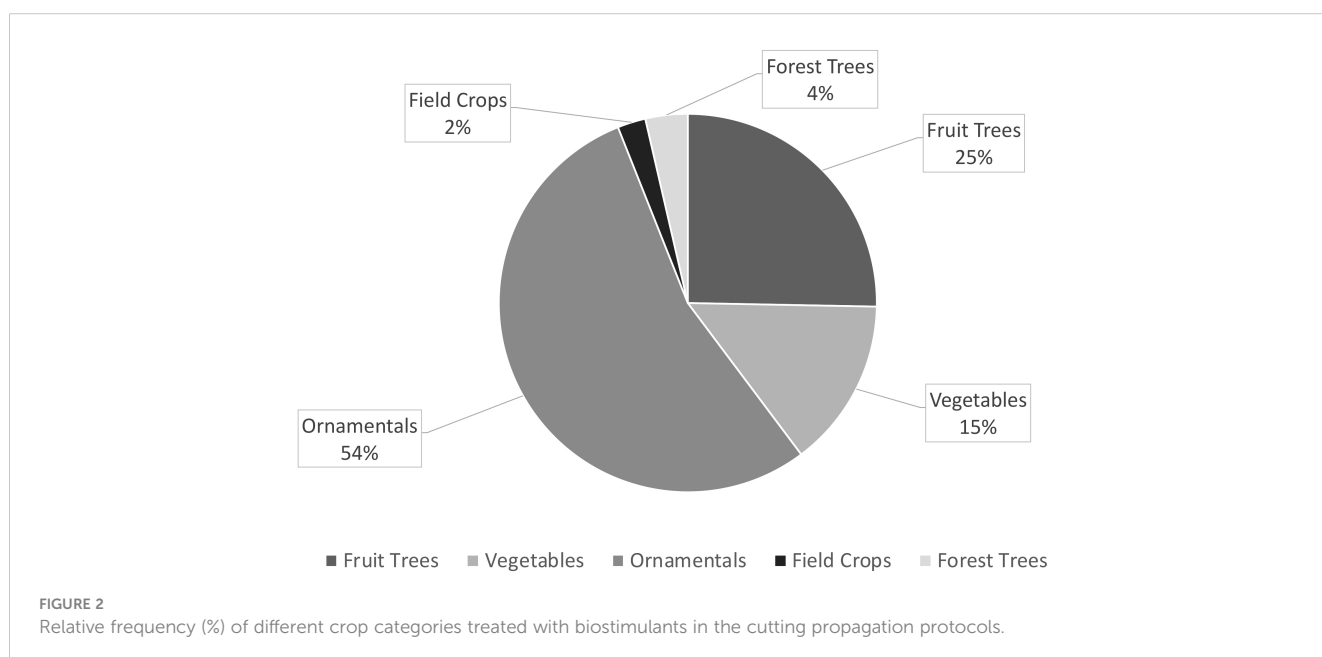


improving leaf development and flowering (Mukherjee and Patel, 2019; Valverde et al., 2022).

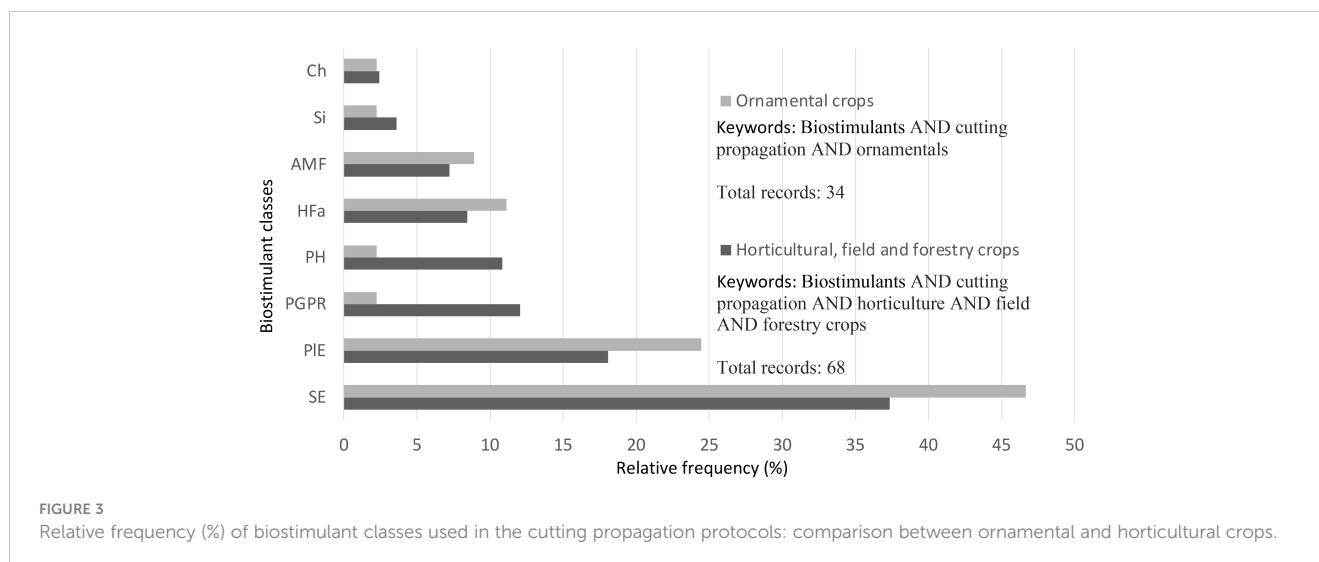
It is crucial to emphasize that biostimulants derived from SEs do not constitute a homogeneous category of products. The characteristics of SE can vary significantly based on factors such as the family and species of seaweed utilized in the manufacturing process (e.g., brown, green, or red seaweed), the source of the seaweed raw material, and the specific extraction methods employed (Fletcher et al., 2017). Commercial brown SEs are a variable mixture of *Ascophyllum nodosum*, *Ecklonia maxima*, *Fucus*, *Laminaria*, *Sargassum*, and *Turbinaria* spp. (Craigie, 2011; Sharma et al., 2012; Górká et al., 2018). Biostimulants derived from *A. nodosum* extract have demonstrated various positive effects on fruit and vegetable

crops: plant vigor, increased root development, enhanced chlorophyll synthesis, promotion of earlier flowering, enhancement of fruit set and uniformity, delayed senescence, and increased tolerance to abiotic stress (Shukla et al., 2019). SE application can introduce microbial communities at the point of inoculation; on the other hand, foliar application to vegetative growth stages is aimed at stimulating signal tracts to reduce abiotic stresses (Adedayo and Babalola, 2023; Krawczuk et al., 2023). These beneficial outcomes highlight the potential of *A. nodosum* extract biostimulants in contributing to overall plant health and productivity (Łangowski et al., 2019).

In terms of biological activity on plants, the main known elicitors from seaweeds are cell wall polysaccharides that also contain a wide range of organic and inorganic molecules that are







known to contribute to their biostimulant activity (Magnabosco et al., 2023). Their application helps in promoting physiological actions like photosynthesis, nutrient metabolism, enzymatic activities, chlorophyll, and carbohydrate content. And consequently, improve the rooting process. It is known that during rhizogenesis, carbohydrates are an important source of energy for plant tissues. Furthermore, a significant portion of sucrose is converted into starch, which is believed to be the primary carbon source for the growth of AR (Lohr et al., 2017).

## 5 Commercial SE-based products as an example

Analysis of the 21 references (Supplementary Table 1) showed that there were 10 commercial SE-based products used in the trials.

Supplementary Figure 1 shows that, in our reference list (n=21), the most widely used commercial SE-based products in the propagation of ornamental plants turn out to be Goteo (23%), Algaminoplant (19%), Bio Rhizotonic (19%), Kelpak (15%) and others (4%). Supplementary Figure 2 illustrates the distribution of other commercial biostimulant categories, non-SE-based: Bio Roots and RootJuice (Ple) are the those with the highest percentage of use. Among phyto regulators, IBA is the most widely used in cutting propagation (60%), followed by NAA (25%) (Supplementary Figure 3). The dose indicated on the label of the following commercial SE-based products is missing for the ornamental crops: Goteo<sup>®</sup>, Kelpak<sup>®</sup>, Algaminoplant, BioRhizotonic.

Goteo<sup>®</sup> (Goteo – Goactiv, UPL, Cesena, Italy) is a commercial liquid seaweed-based biostimulant, containing GA142, a filtrate from the seaweed *A. nodosum*, a source of auxins and cytokinins, polysaccharides and vitamins (Stępowaska, 2008; Francke et al., 2022). In the preparation, GA142 is added with organo-mineral fertilizers (w/v): 13% P<sub>2</sub>O<sub>5</sub>, 5% K<sub>2</sub>O, and 1.3–2.4% organic substances (Loconsole et al., 2022b).

Kelpak<sup>®</sup> (Kelp Products Pty Ltd, Simon's Town, South Africa) (Table 2) is a liquid concentrate derived from the stipes and laminae

of the brown kelp, *E. maxima* (Osbeck) Papenfuss (Crouch and Van Staden, 1991). It is commercially used as a plant growth stimulator reducing nursery periods before out-planting and increasing the yield and quality of a variety of terrestrial crops (Crouch and Van Staden, 1991; Al-Hawezy, 2015; Kocira et al., 2018). It is also known to aid in producing stronger and healthier crops by enhancing root formation and reducing transplant shock resistance.

Algaminoplant (Varichem, Poland), is a liquid preparation composed of SE derived from *Sargassum*, *Laminaria*, *Ascophyllum*, and *Fucus* at 18% concentration. It incorporates phytohormones with gibberellin-like activity equivalent to 0.005% gibberellic acid (GA3), cytokinin activity equivalent to 0.0005% benzyl adenine (BA), and auxin-like activity corresponding to 0.003% IAA. Additionally, it is enriched with potassium salts of amino acids at a concentration of 10% (Matysiak et al., 2010).

Bio Rhizotonic (Canna Continental, Los Angeles, USA), identified as an algae-based rooting liquid stimulator, includes vitamins such as B1, B2, and N-P-K at a ratio of 0.6–0.2–0.6 (Monder and Pacholczak, 2020).

Actiwave<sup>®</sup> (Valagro SpA., Atessa, Italy) is a SE product derived from *A. nodosum* and its three major components are kahydrin, alginic acid, and betaine. Its composition includes organic carbon (12%), organic N (1%), Total N (3%), N ureic (2%), K<sub>2</sub>O (7%) at 6.4 pH. The dose indicated on the label is 300–500 mL 100 L<sup>-1</sup> for the ornamental crops.

TABLE 2 Composition of commercial SE Kelpak<sup>®</sup> (34% w/w *E. maxima*).

IBA: 11.16 mg L <sup>-1</sup>	N: 0.09%	Mn: 17.3 mg Kg <sup>-1</sup>
Cytokinin: 0.031 mg L <sup>-1</sup>	P: 90.7 mg Kg <sup>-1</sup>	Fe: 40.7 mg Kg <sup>-1</sup>
Alginates: 1.5 L <sup>-1</sup>	K: 7163.3 mg Kg <sup>-1</sup>	Cu: 13.5 mg Kg <sup>-1</sup>
Amino acids: 441.3 mg 100 g <sup>-1</sup>	Ca: 190.4 mg Kg <sup>-1</sup>	Zn: 17.0 mg Kg <sup>-1</sup>
Mannitol: 2261 mg L <sup>-1</sup>	Mg: 337.2 mg Kg <sup>-1</sup>	B: 33.0 mg Kg <sup>-1</sup>
Neutral sugars: 1.08 g L <sup>-1</sup>	Na: 1623.7 mg Kg <sup>-1</sup>	

Adapted from Szczepanek and Siwik-Ziomek (2019).

## 6 Applied protocols with commercial SE-based products

‘The ability to demonstrate that a product is indeed *a bona fide* biostimulant will depend on a demonstration of its effect’ (Ricci et al., 2019).

### 6.1 Goteo®

Goteo® has been observed to stimulate the greenhouse development of the *Ornitogalum* bulb’s twin scales, excised from the outer and inner of the parent bulb and soaked for 30 minutes in 0.2% solution of Goteo® (Salachna et al., 2014). The study revealed that exciting twin scales from the outer layer of the parent bulb led to a greater formation of adventitious bulbs that exhibited higher fresh weight and circumference, as well as a greater number and length of roots.

Although not in a greenhouse protocol comparing SE-based biostimulants with IBA, Kapczynska et al. (2020) found that in *Pennisetum* ‘Vertigo’ cuttings Goteo®, at a dose of 0.1%, stimulated AR development, having a significant impact on root elongation, regardless of whether peat or perlite was used as the rooting medium. Watering the cuttings with Goteo® 5 times every 5 days proved to be highly effective, achieving a 100% success rate, compared to soaking the 2 cm cuttings base for 20 min and applying foliar Goteo spraying 5 times every 5 days. For each Goteo treatment, a control was set up in which the cuttings were treated as follows: soaking with water, watering with water and spraying with water. Furthermore, Goteo® watering stimulated the generation of new shoots during greenhouse cultivation. The application of Goteo® resulted in an increased phosphorus and potassium content. Khan et al. (2009) observed a beneficial effect of plant P nutrition because of the application of SE-based biostimulants.

Loconsole et al. (2022b) investigated the effects of Goteo® compared to IBA application, in the process of rhizogenesis of two ornamental shrubs, *Lantana camara* and *Abelia x grandiflora*, in a propagation greenhouse. The treatments applied to semi-hardwood stem cuttings were as follows: control (distilled water as spray); IBA (Sigma, St. Louis, MO, USA) solution at concentration 1,250 mg L<sup>-1</sup>; Goteo® concentrations at 1, 2, and 3 mL L<sup>-1</sup> as spray application at two-week intervals, totaling three applications. Regarding the concentration, the company recommends 0.1% solution (1 mL L<sup>-1</sup>) on vegetable crops and does not provide a dosage for ornamentals. On the other hand, Gajc-Wolska et al. (2012) recommended the dosage for hastening the rooting system regeneration as being equal to three to four treatments with a 0.2% solution (2 mL L<sup>-1</sup>) every 2 weeks. SE biostimulant was found to be effective in enhancing rooting percentage as well as in root architecture.

Findings revealed that Goteo® at a dose of 3 mL L<sup>-1</sup> in wild sage and 1 mL L<sup>-1</sup> in glossy abelia, Goteo® produced similar results to those obtained with IBA regarding rooting percentage (in lantana: IBA= 91%, Goteo® 3 mL L<sup>-1</sup> = 87%; in abelia: IBA= 93%, 1 mL L<sup>-1</sup> =

90%). Moreover, the stimulator was successful in promoting the growth of many roots and enhancing architecture parameters when compared to IBA. Better results were found in wild sage at a dose of 3 mL L<sup>-1</sup> compared to IBA treatment, regarding root length (+27%), surface area (+47%), diameter (+22%), number of tips (+41%), forks (+61%), and crossings (+31%). In *A. x grandiflora*, on the contrary, the applications of IBA and Goteo® 1–2 mL L<sup>-1</sup> determined the greatest root length (391, 396 and 336 mm, respectively) and root surface area values (67, 70 and 61 mm<sup>2</sup>, respectively).

In addition to observing that SE-based biostimulant had a beneficial effect on AR and shoot quality of cuttings, Pacholczak and Nowakowska (2020) also noted, in two ground cover roses ‘Elfrid’ and ‘Weisse Immensee’ under a plastic tunnel, those on both chlorophyll contents (chlorophyll a+b) and soluble sugars (Supplementary Figures 4, 5). The goal of their experiment was to compare the effects of Goteo® and Algaminoplant, applied at a concentration of 0.2% for foliage spraying, to IBA (Rhizopon 1% or water solution 200 mg L<sup>-1</sup>). Algaminoplant and Goteo® treated cuttings, compared to the control, led to an increase in chlorophyll content by 23% and 28% in Elfrid and by 19% in Weisse Immensee, respectively. Compared to untreated cuttings, those treated with Goteo® (0.2%) had the highest values of total soluble sugars and chlorophyll compared to those treated with Rhizopon (1% IBA) or water solution.

Monder et al. (2014) and Monder and Pacholczak (2018) in rose cuttings showed the positive impacts of IBA and SE-based biostimulants on sugar levels, vital for effective rhizogenesis since the effectiveness of photosynthesis in cuttings is low. AR formation is a highly energetic process; the capability of young cutting to produce carbohydrates both as storage starch and ready-to-use simple sugar forms is fundamental.

### 6.2 Kelpak®

Leafy shoot cuttings are exposed to several environmental stresses during rooting. Szabó et al. (2011) tested, on stockplants of *Prunus* ‘Marianna 8-1’ raised in the open field, two biostimulants: Kelpak® (0.2%) and Wuxal Ascofol® (0.2%, containing *A. nodosum* extract) a PGPR-based biostimulant, Pentakeep®V (0.03%), containing 5-amino-levulic-acid, and untreated control. applied three times before cutting. Kelpak® and Wuxal Ascofol® increased the number and the weight of shoots, the weight of cuttings and the leaf chlorophyll content. Therefore, shoots of treated stock plants reached earlier the optimum size for cutting propagation. Application of Kelpak® resulted in the highest rooting rate and increased the fresh weight of cuttings during rooting surpassing both control and cuttings treated with the other two preparations tested.

Maintaining green, functionally active leaves during root induction is crucial since root formation depends on adequate carbohydrate supply from the cutting’s source leaves to the region of root regeneration.

Traversari et al. (2022), aimed to test the replacement of synthetic phyto regulators, such as auxins and brassinosteroids, with a commercial SE, for AR formation, analyzed in greenhouse,

‘Michelangelo’<sup>®</sup> and ‘Cosmos’<sup>®</sup>, rose cuttings, tested with five different treatments: distilled water (control), 10% Kelpak<sup>®</sup> and 10% Phylgreen in distilled water, 4000 ppm IBA and NAA in distilled water; 5 ppm of 22(S),23(S)-homobrassinolide in distilled water. Results showed that Kelpak<sup>®</sup> improved rooting percentage and root biometric parameters in both cultivars. Moreover, root fresh weight was significantly higher under both auxin and Kelpak<sup>®</sup> treatments, with increases of +99% and +62%, respectively. Additionally, root length demonstrated notable enhancements under auxin and Kelpak<sup>®</sup> treatments in the ‘Michelangelo’<sup>®</sup>, with increases of +103% and +75%, respectively, compared to the control.

Loconsole et al. (2023) observed that, in *Photinia x fraseri* ‘Red Robin’ cuttings, Kelpak<sup>®</sup> and Goteo<sup>®</sup> at the doses of 2 and 3 mL L<sup>-1</sup> applied as foliar spray four times in greenhouse propagation, stimulated the production of callus in over 80% of cuttings treated, whereas IBA produced the highest rooting percentage. Moreover, it appears that the abundant callus tissue production hinders the rooting, creating a structural barrier to the development of AR. These results are consistent with those of Monder and Pacholczak (2017) and Monder et al. (2021), who found that in the rhizogenesis of the ‘Hurdal’ rose, an increase in rooting percentage was only observed when the percentage of cuttings with callus decreased.

### 6.3 AlgaminoPlant and Bio Rhizotonic

Pacholczak et al. (2017) reported a comparable effect on the rooting potential of cuttings of *Cornus alba* ‘Aurea’ and ‘Elegantissima’, treated with AlgaminoPlant at 0.2% water solution with cuttings treated with Rhizopon AA (1% IBA) and IBA 200 mg L<sup>-1</sup>.

Pacholczak et al. (2016b) reported a beneficial impact of AlgaminoPlant (0.2%), on the rooting of ninebark cuttings (*Physocarpus opulifolius*), sprayed once, twice or three times during the rooting period, compared to Rhizopon AA (2% IBA) and a water solution of 200 mg dm<sup>-3</sup> IBA. The control cuttings were sprayed with distilled water. Two nodal stem cuttings were rooted in styrofoam boxes with a mixture of peat, perlite and sand in a greenhouse propagation. The trial was repeated in two years (2012 and 2013). A triple treatment with AlgaminoPlant was observed to increase the percentage of rooted cuttings by 36% (2012) and 26% (2013) compared to the control and showed comparable or slightly weaker effects to the treatments with synthetic IBA.

Pacholczak and Nowakowska (2020) compared the effects of AlgaminoPlant, Goteo<sup>®</sup> and Rhizopon (1% IBA) on cuttings in two ground cover roses ‘Elfrid’ and ‘Weisse Immensee’ growth under a plastic tunnel. SEs were applied by foliar spraying at a concentration of 0.2%. The experiment’s findings revealed that AlgaminoPlant exerted a positive influence on rhizogenesis in both cultivars, comparable to or only slightly less potent than those induced by the IBA. Furthermore, the application of Goteo<sup>®</sup> led to an increase in the content of chlorophyll and total soluble sugars in cuttings. On the other hand, the levels of free amino acids and polyphenolic acids were reduced under the influence of Goteo<sup>®</sup>.

The research carried out by Monder et al. (2021) focused on the response of stem cuttings of Rosa ‘Hurdal’ treating the cuttings with

Bio Rhizotonic and showed that the anatomical structure of cuttings was influenced by plant-derived biostimulants.

Historical roses are especially difficult to propagate, with the process of rhizogenesis lasting for a minimum of 12 weeks and connected with low quality of rooted cuttings. Monder and Pacholczak (2023) pointed out that, on six aged, once-flowering rose cultivars (‘Duchesse d’Angoulême,’ ‘Hurdals,’ ‘Maiden’s Blush,’ ‘Mousseuse Rouge,’ Rosa beggeriana ‘Polstjärnan,’ R. helenae ‘Semiplena’) treated with Bio Rhizotonic, the polyphenolic acid content decreased during the rhizogenesis period.

### 6.4 Other SE-based products

Ferrante et al. (2012), pointed out an experiment on cuttings of *Camellia japonica* L. using Actiwave<sup>®</sup> (Valagro Spa) at the doses of 0.015 mL and 0.03 mL per application, for a total of eight applications per month, directly on the substrate. The effect of the biostimulant on the rooting of cuttings was evaluated by comparing it with applications of gibberellic acid (GA<sub>3</sub>) at doses of 1.25 mg and 2.5 mg. These doses were administered at identical intervals of Actiwave<sup>®</sup>, with nebulization on both the leaves and the substrate of the cutting, resulting in a cumulative application of 10 and 20 mg. After 127 days, the rooting percentage for cuttings treated with 0.12 mL/L Actiwave<sup>®</sup> was 82%, contrasting with the control group, which exhibited a rooting percentage of 18%. By the end of the experiment (161 days), the rooting value for the Actiwave<sup>®</sup>-treated cuttings further increased to 88%, while the control group showed a rooting percentage of 55%.

Toța et al. (2022) carried out a study on *Crassula ovata* clonal propagation, comparing several biostimulants, including Raiza, a liquid product containing bioactive substances extracted from *A. nodosum*, 20 free amino acids including proline and serine, phytohormones including cytokinins, auxins and gibberellins. The experimental protocol lacks in SE doses and number of applications.

Abdel-Rahman et al. (2020) assessed the impact of a commercial SE-based biostimulant (Tera at 3 mL L<sup>-1</sup> or 10 mL/pot), IBA, both independently and in combination with PGPR (*Agrobacterium rhizogenes*) at 10<sup>8</sup> CFU mL<sup>-1</sup>, and Ple biostimulants (coconut water), on the root quality of cuttings in *Conocarpus erectus* L. The treatments were administered as follows: for IBA, at 100 ppm, the basal ending of cuttings was soaked for 20 hours at the start of the trial; PGPR were inoculated in the rooting substrate before sticking cuttings; Tera was sprayed (3 mL L<sup>-1</sup>) or drenched (10 mL pot<sup>-1</sup>) once every 5 days for three times in total. Finally, for coconut water, the basal ending of the cuttings was soaked for either 3 min or 1 h before planting.

The findings revealed that all treatments involving IBA and/or biostimulants significantly increased the rooting percentage, as well as the root and vegetative characteristics of the rooted cuttings when compared to untreated cuttings. Notably, the individual applications of seaweed extract and coconut water demonstrated greater efficacy than IBA or *A. rhizogenes* alone. Additionally, treatments involving seaweed extract as a drench, with or without IBA, outperformed those of seaweed extract treatments as a spray.

Gomes et al. (2018) tested a commercial SE product (name not given) manufactured by Acadian Seaplants<sup>®</sup> in the greenhouse, to



assess its impact when applied to the bases of *P. actinia* stem cuttings. Five concentrations of the extract in distilled water were tested: 0, 10, 20, 30, and 40%. Stem cuttings were dipped for 2 min in solution before planting. On average, a rooting percentage of 51% was achieved. The rooting percentage exhibited a linear increase in correlation with the concentrations of the brown seaweed extract. In comparison to the control treatment, a notable 10% increase in rooting was observed with the 40% seaweed extract treatment.

## 7 Conclusions

The ornamental's worldwide production has increased in recent years due to their great use in urban parks and green areas, ensuring ecosystem services. Despite significant advancements in stem-cutting propagation, a complicated process that starts from high-quality rooted cuttings, insufficient rooting efficiency remains an economic burden for the ornamental nursery industry. Synthetic phyto regulator as IBA, with a high carbon footprint, when applied exogenously to cutting, however, enhances AR formation.

In sustainable agriculture, the substitution of chemical inputs, with alternative natural eco-friendly products presents a key challenge. Biostimulants, renewable sources, can form part of a solution to mitigate such risks deriving from the use of agrochemicals.

This review shows the positive effects of the main commercial SE-based products on AR formation and quality of rooted cutting, highlighting their effectiveness on rooting percentage, root number and architecture. Moreover, they increase the overall quality of a rooted cutting as dry biomass and organic compound content.

The findings show that the effects, unfortunately, are species-specific and product-specific, depending on: (i) the type of seaweed resource; (ii) the quality and composition of the extract; (iii) the method, concentration, and frequency of application.

Especially, when the raw material comes from macroalgae, it turns out to be extremely complex, to identify the numerous components exerting a role in the biostimulation mechanism. Despite the intricate nature of this product, identification of its bioactive ingredients is critical to building trust and credibility in its commercialization.

To conclude, it is possible to state that:

- The same dose can prove effective if administered with one SE commercial product and ineffective with another (i.e. Kelpak vs Phylgreen);
- The same dose should be optimized with the species and cultivar (i.e. Goteo<sup>®</sup> at a dose of 3 mL L<sup>-1</sup> in wild sage and 1 mL L<sup>-1</sup> in glossy abelia);
- The optimal dose depends on the growing season too: as occurred in 'GiSelA 5' softwood cuttings;
- SEs as a drench with or without IBA surpassed those of SE treatments as a spray (i.e. in *C. erectus* cuttings);

- Where no relationships exist between the type of cutting (herbaceous or woody, top or branch) and the most suitable biostimulants, need to operate empirically.

This review also shows that the application of SE-based biostimulants in replacement of IBA can ensure results of comparable quality in ornamental cutting propagation.

Therefore, it will be necessary to create newly standardized protocols to validate and give credibility to the effects of SE-based biostimulants applied to clonal multiplication.

The specific application of other biostimulants, such as AMF and PGPR, can be useful in the sector of ornamentals to pursue research.

## Author contributions

DL: Conceptualization, Writing – original draft, Writing – review & editing. ES: Writing – original draft. AS: Writing – original draft. GC: Writing – original draft, Writing – review & editing. BD: Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fhort.2024.1371090/full#supplementary-material>

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