

Effect of Biostimulants on Rooting Quality in Ornamental Cuttings

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Modern Vegetative Propagation

Introduction

Greenhouse Technology & Controlled Propagation

- 1950s: intermittent mist & plastic film
 - 1980s: fog systems and bottom heat
- 1970-90s: crop commercial micropropagation



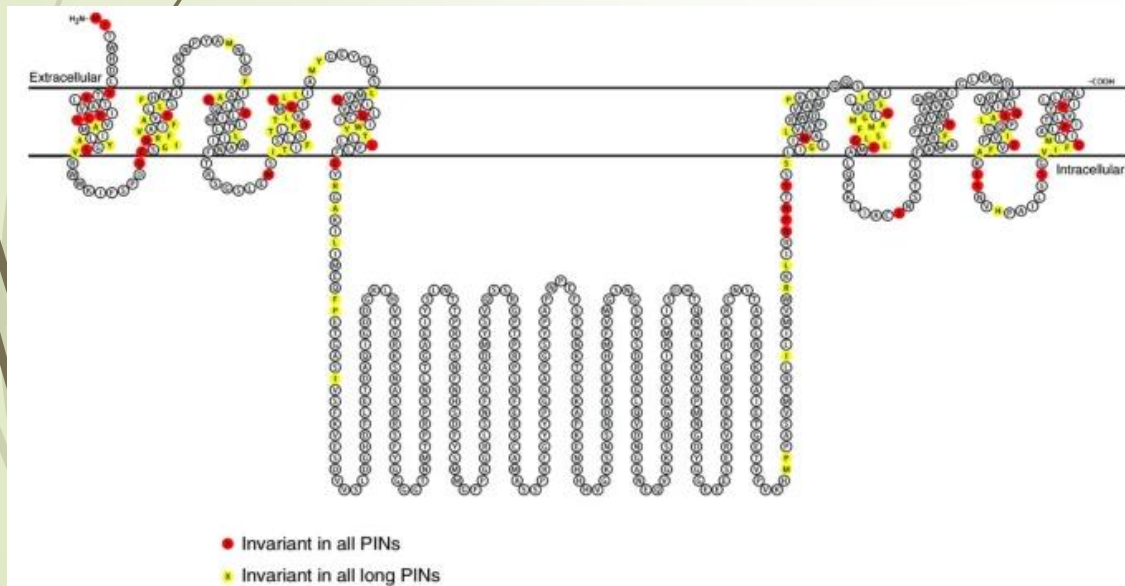
1990-2000

Introduction

PIN formed proteins

Proteins that "point" or "anchor" the direction of auxin flow

Their discovery in the 1990s and the understanding of their mechanism in the 2000s **revolutionized plant developmental biology and clonal propagation techniques.**



Adventitious rooting depends on the basal accumulation of endogenous auxins (IAA) transported polarly from the apex

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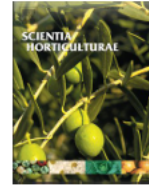
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Research Paper

Influence of water spraying intervals and indole-3-butyric acid concentrations on *Salvia* rooted cuttings quality in a closed aeroponics system

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Rhizogenesis in woody cuttings:
Treatments with **exogenous auxin**
to overcome the ABA/GA barrier

ABA: Maintains cambium and parenchymal cells in cell cycle arrest (G1 phase block), preventing the dedifferentiation necessary for root callus formation.

GA: Genetically antagonizes root growth.

Introduction

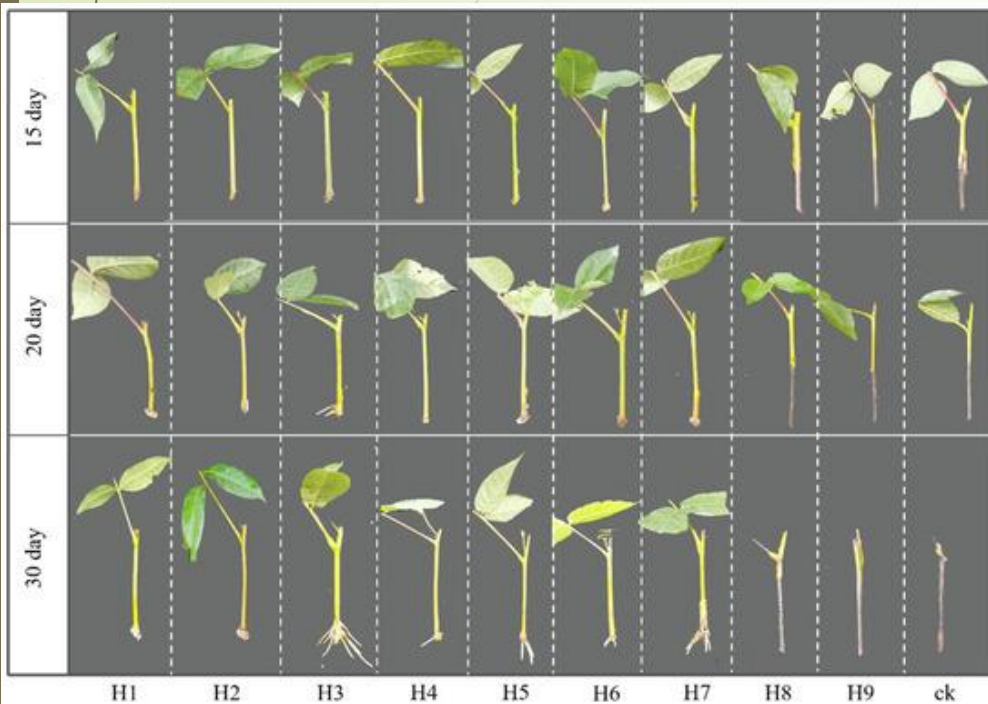
- Global ornamental plant market: >\$50 billion annually with vegetative propagation as primary clonal method
- Critical bottleneck: Adventitious root (AR) formation in recalcitrant ornamentals (rose, lavender, chrysanthemum, barberry)
- Regulatory pressure: Increasing restrictions on synthetic auxins (IBA/NAA) in organic systems
- EU Regulation 2019/1009 defines biostimulants as products stimulating plant nutrition processes independently of nutrient content
- Key distinction: Biostimulants act via physiological modulation, not mineral nutrition—distinct from fertilizers

Beyond the application of auxins

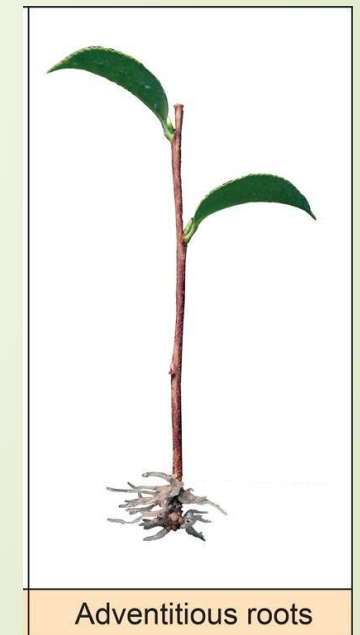
Introduction

Biostimulant-Mediated Adventitious Root Formation

Phytohormonal Mechanisms, Species-Specific Responses,



Phase 2: Initiation (Root primordia formation)



Phase 3: Elongation (Root growth)

The aim of the research in
this focus

Redefining Rooting Quality

Metrics:

rooting percentage
root number,
root length, ...

"Root quality determines post-transplant survival and establishment" (Dawson et al., 2021, *Horticulture Research*, 8: 156. <https://doi.org/10.1038/s41438-021-00589-3>)

Emerging indicators:

Integrative Indicators of Rooting Success

Morphological quality:

Root system architecture (RSA) analysis via WinRHIZO or similar

Physiological quality:

Non-structural carbohydrates (NSC): starch, soluble sugars

Hormonal balance (auxin-cytokinin ratio)

...

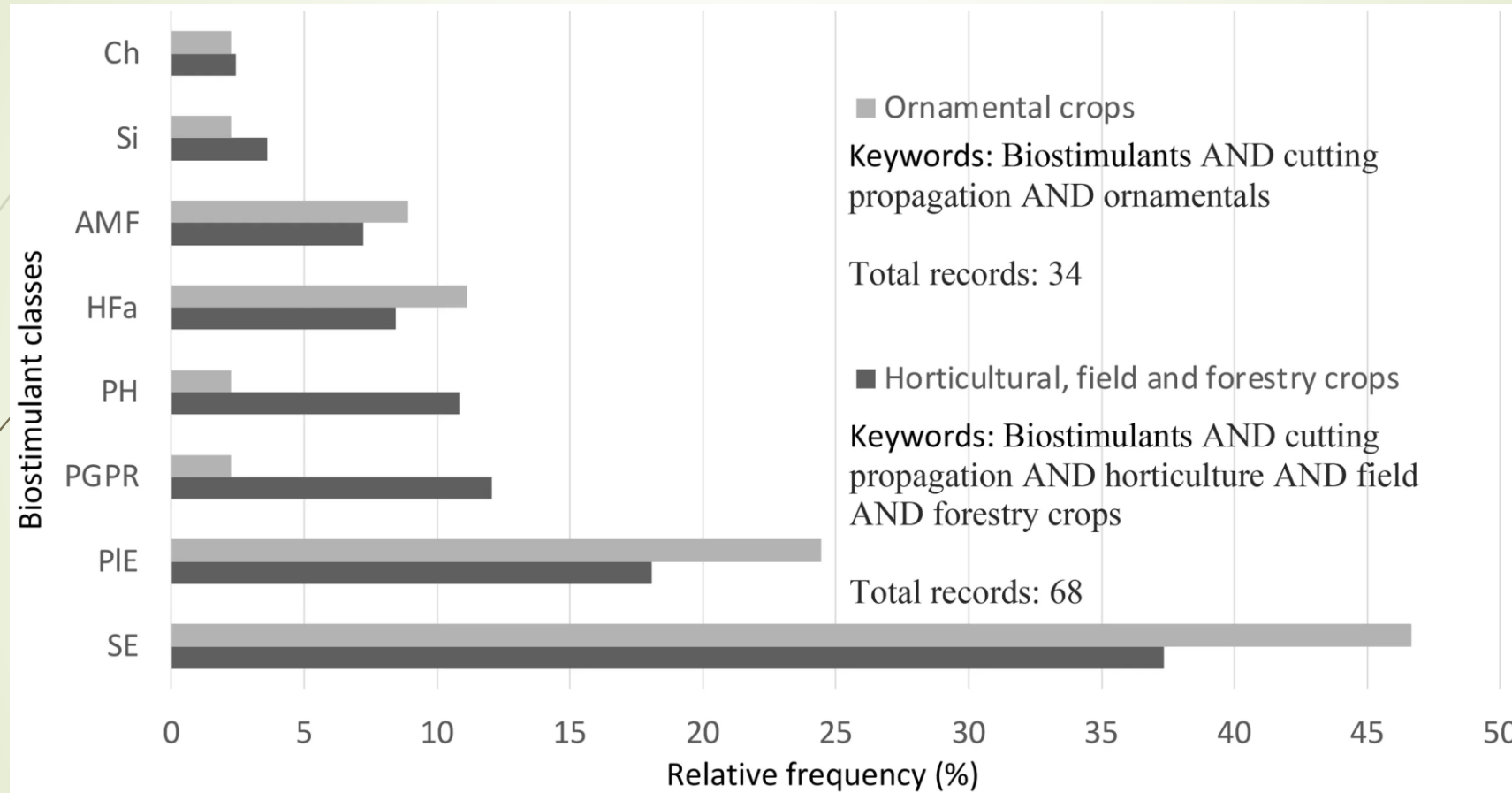
Biochemical quality:

Antioxidant capacity

...

"Root quality determines post-transplant survival and establishment" (Dawson et al., 2021, *Horticulture Research*, 8: 156. <https://doi.org/10.1038/s41438-021-00589-3>)

Relative frequency (%) of **biostimulant classes** used in the cutting propagation protocols: comparison between ornamental and horticultural crops.



SE Seaweed extracts;

PIE Plant extracts,

PGPR beneficial bacteria,

PH protein hydrolysates;

HFa humic acid and fulvic acid;

AMF beneficial fungi;

Si silicon;

Ch chitosan and other biopolymers

Loconsole, D., Scaltrito, E., Sdao, A. E., Cristiano, G., & De Lucia, B. (2024). Application of commercial seaweed extract-based biostimulants to enhance adventitious root formation in ornamental cutting propagation protocols: a review. *Frontiers in Horticulture*, 3, 1371090.

Seaweed Extracts

Hormone-like activity

Composition: Cytokinins (zeatin, kinetin), auxins, GAs, ABA, BRs, jasmonates; polysaccharides (alginates, laminarin); betaines

- *Ecklonia maxima* on Rosa 'Michelangelo': 77% rooting (+19% vs. control); +62% root fresh weight; +75% root length
- *Ascophyllum nodosum* on ground cover roses: Enhanced chlorophyll and soluble sugars; reduced free amino acids vs. IBA
- Application: Foliar spray or cutting immersion; concentration range 0.2-3 mL L⁻¹ species-dependent

Morphological quality:

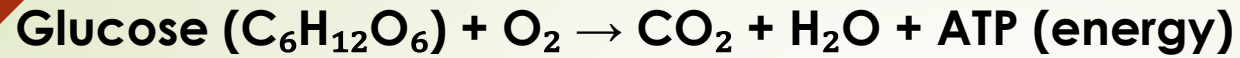
Table 5. Root morphological traits: root tips (no.), forks (no.) and crossings (no.) in *Lantana camara* (S1) and *Abelia × grandiflora* (S2), influenced by rooting promoters at different concentrations (C).

TMTS	Root					
	Tips (No.)		Forks (No.)		Crossings (No.)	
	S1	S2	S1	S2	S1	S2
CO	45 ± 2 d	32 ± 3 c	197 ± 4 d	79 ± 3 c	30 ± 3 c	13 ± 1 c
IBA	97 ± 6 c	73 ± 3 a	430 ± 11 c	239 ± 4 a	81 ± 1 b	38 ± 1 a
GC1	114 ± 5 b	68 ± 9 a	477 ± 14 c	242 ± 11 a	83 ± 3 b	38 ± 1 a
GC2	116 ± 6 b	37 ± 3 c	613 ± 35 b	136 ± 3 b	84 ± 4 b	23 ± 2 b
GC3	137 ± 5 a	53 ± 2 b	694 ± 16 a	128 ± 5 b	106 ± 2 a	19 ± 1 b

Different letters mean significant differences within parameters (S.N.K. test, $p \leq 0.05$; mean \pm SD, $n = 3$). IBA: Indole-3-Butyric Acid; G: commercial seaweed extract Goteo[®]; CO, untreated control; IBA: 1250 mg L⁻¹; GC1: 1 mL L⁻¹; GC2: 2 mL L⁻¹; GC3: 3 mL L⁻¹.

Physiological quality

Non-structural carbohydrates (NSC)



Energy-demanding processes required:

- Protein synthesis (enzymes)
- Cell division (mitosis)
- Nucleic acid synthesis
- Hormone production (auxin)
- Active transport (membranes)
- Stress defense (osmotic)



Adventitious root formation

Carbohydrate reserves are essential until roots become functional

Low soluble sugars reduce rooting capacity regardless of auxin treatment

(Hartmann et al., 1979)

Biostimulants	Species	Effect on sugars	Consequence
Humic acids	Rice	↑ + 27- 74% soluble sugars	↑ Energy for rooting
Protein hydrolysates	Basil	↑ Carbohydrate reserve	↑ Post-transplant survival
Seaweed extracts	Grapevine	↑ Starch and sugars	↑ Rooting percentage

Table 6. Starch content ($\text{mg g}^{-1}\text{d.w.}$) in *Lantana camara* (S1) and *Abelia* \times *grandiflora* (S2), influenced by rooting promoters at different concentrations (C).

TMTS	Starch	
	S1	S2
C0	2.43 \pm 0.26 b	6.93 \pm 0.03 b
IBA	3.36 \pm 0.03 a	6.23 \pm 0.17 c
GC1	3.03 \pm 0.09 a	7.30 \pm 0.06 a
GC2	3.00 \pm 0.11 a	7.40 \pm 0.01 a
GC3	3.13 \pm 0.03 a	6.90 \pm 0.06 b

Different letters mean significant differences within parameters (S.N.K. test, $p \leq 0.05$; mean \pm SD, $n = 3$). IBA: Indole-3-Butyric Acid; G: commercial seaweed extract Goteo[®]; C0, untreated control; IBA: 1250 mg L^{-1} ; GC1: 1 mL L^{-1} ; GC2: 2 mL L^{-1} ; GC3: 3 mL L^{-1} .






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Article

Improving Aerial and Root Quality Traits of Two Landscaping Shrubs Stem Cuttings by Applying a Commercial Brown Seaweed Extract

Danilo Loconsole , Giuseppe Cristiano  and Barbara De Lucia 

Hormonal Balance Model

In ornamental cuttings treated with biostimulants, the enhanced rooting is achieved through:

- ✓ Direct hormone provision; auxin stimulation and biosynthesis
- ✓ BR-mediated enhancement (phytosterol precursors → active brassinosteroids)

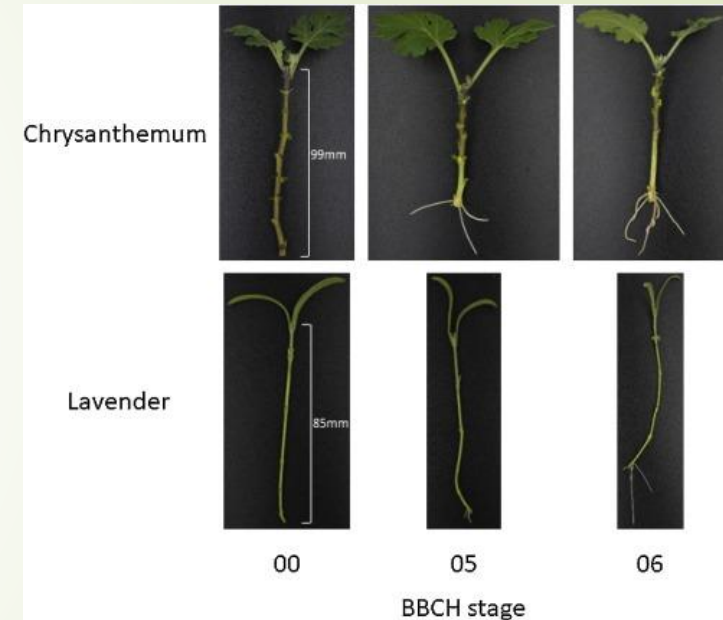
Kelp + *Aloe vera* extracts (P1) and Willow bark extract (P2)

Cuttings from lavender (soft wood) and chrysanthemum (semi-hard wood).

Application of P1 at 1 mL/L concentration achieved consistent improvements in the formation of adventitious roots and root branching in both lavender and chrysanthemum.

Application of P2 at a concentration of 1.06 $\mu\text{L/L}$ yielded the best results for both species. It promotes root branching more than adventitious root formation.

Advantage: accelerates the propagation of soft and semi-hard wood cuttings



Wise, K., Gill, H., & Selby-Pham, J. (2020). *Scientia Horticulturae*, 263, 109108.

Protein Hydrolysates (PH)

Tryptophan: The IAA Precursor

Tryptophan is the main precursor of **indole-3-acetic acid (IAA)** in plant biosynthesis pathways. PH contains high tryptophan content (**1.4%**), which presumably explains the auxin-like activity.

Biosynthesis Pathway:

Tryptophan → IAA → Root Growth

Bioactive Peptides

In addition to tryptophan, **certain peptides** from plant-derived PHs can directly induce IAA-like activity. Matsumiya identified a **root hair-promoting peptide** from degraded soymeal products.

Key Finding:

Specific peptide sequences can mimic auxin signaling, triggering cell elongation and root hair formation independent of tryptophan content.

Dual Mechanism of Auxin-Like Activity

A Tryptophan-Mediated Pathway

- Direct precursor for IAA synthesis
- 1.4% content in PH
- Enhances endogenous auxin production
- Promotes cell elongation

B Peptide-Direct Signaling

- Bioactive peptide sequences
- Mimic auxin receptor binding
- Trigger downstream responses
- Root hair promotion

Biostimulant Induces Adventitious Rooting of Stem Cuttings Primarily via BR-Mediated Processes

Kim, H. J., Ku, K. M., Choi, S., & Cardarelli, M. (2019)

Brassinolide, 24-epibrassinolide and 28-homobrassinolide are used as rooting enhancers

Biostimulant Composition:

Plant-derived protein hydrolysate (vegetal-derived)

Contains 4.1% tryptophan (precursor for auxin biosynthesis)

Contains brassinosteroid precursors:
Campesterol and Stigmasterol

The study demonstrated that the vegetal-derived biostimulant enhances the antioxidant defense system during adventitious rooting, which is crucial for:

Protecting auxins from decarboxylation

Reducing oxidative stress caused by wounding during cutting preparation

Promoting root formation through BR-mediated processes

C. indicum: Vegetal-derived biostimulant increased root dry mass logarithmically (optimal: 5000 mgL⁻¹)

The Brassinosteroid Concentration Paradox

When Less is More: The Biphasic Response of BR-Mediated Rooting

Biphasic response: stimulation (nM- μ M) vs. inhibition ($>10 \mu$ M)

Mechanism: BZR1 hyperphosphorylation \rightarrow activation of stress genes

The same substance (BR) can be a "drug" or a "poison" depending on the dose

Recent data:

Rosehip: optimal at 0.5 ppm (Çeliker & Koç, 2022)

Berberis thunbergii: requires 500 ppm (1000x higher!)

Species	Dose	Message	Rooting	Root quality
rose	[0,5 ppm BR]	Clear message	Root formation	8-10 healthy roots
rose	[50 ppm BR]	Too many messages! Panic!	No roots	Burned tissue; death of the cutting

Çeliker, M., & Koç, A. (2022). Effect of IBA, Brassinosteroid, and Bacterial Applications on Rooting of Some Rosehip (*Rosa canina* L.) Genotypes by Hardwood Cuttings. *Journal of the Institute of Science and Technology*, 12(2), 539-549.

The Concentration Paradox Revisited

Species-Specific BRs Dose Optimization and the Biphasic Response

Species	Optimal [BR]	Response type	Reference
<i>Arabidopsis thaliana</i>	0.001–0.1 nM	Biphasic (inhibition >100 nM)	Kim et al., 2006
<i>Berberis thunbergii</i>	500 ppm (0.05%)	Linear to optimum	Pacholczak et al., 2021
<i>Rosa canina</i>	0.5 ppm	Linear to optimum	Çeliker & Koç, 2022
<i>Pelargonium</i> spp.	0.5–1.0 ppm	Optimal range	Çeliker & Koç, 2022

The Brassinosteroids application

Factors	Impact on BRs effectiveness
Species	Some species are naturally more sensitive
Type of cutting	Semi-woody > Woody > Herbaceous
Concentration	follow the biphasic response
Application method	Basal immersion > Leaf spray
Combination with auxin	BR + IBA synergy often optimal
Cutting period	More responsive spring cuttings

Conclusion

Biostimulants improve rooting quality in ornamental cuttings through a multi-level action that integrates:

Direct stimulation: auxin-like precursors (tryptophan) and active brassinosteroids

Hormonal modulation: optimisation of the auxin/cytokinin ratio and BR-auxin interaction

Metabolic improvement: increased reserves of non-structural carbohydrates (starch, soluble sugars) essential for the energy phase of root growth

Enhancement of antioxidant defense: BR-mediated increase in root antioxidant capacity, with the opposite effect to exogenous auxin.



Future Perspectives

Characterization of the specific antioxidant profile of woody ornamental species

Optimization of application protocols

Development of customized formulations for different phenological stages of propagation material

Thank you for your kind attention.