

Optimising *Miscanthus* - a clean energy biofuel to reach Net Zero targets

**A thesis submitted in partial fulfilment of the requirements of Liverpool John Moores
University for the degree of Doctor of Philosophy**

by

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Declaration

I declare that the work presented in this thesis is entirely my own and has not been submitted, in whole or in part, for any other degree or qualification at this or any other university or institution of higher education.

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Dedication

This thesis is dedicated to my mother, Agnes Obaje, whose unwavering sacrifice and commitment made my educational journey possible. Despite limited means, she provided steadfast support from basic through tertiary education. Her enduring encouragement and belief have been a constant source of strength.

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“There has never been the slightest doubt in my mind that the God who started this great work in you would keep at it and bring it to a flourishing finish...” Philippians 1:6 (MSG).

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Contents

Lay summary	7
Chapter 1	
General Introduction	10
Chapter 2	
Review: Plant Growth Regulators, Biostimulants, and Microbial Inoculants in Miscanthus: A Scoping Review of Evidence, Experimental Designs, and Research Gaps (2010–2025).	41
Chapter 3	
Can Biostimulants and Phytohormones Improve Early Growth, Biomass Allocation, and Photosynthesis in Miscanthus Plug Plants?	68
Chapter 4	
How Growth Regulators Reshape Miscanthus: Hybrid-Specific Biomass Allocation and Cross-Environment Stability	99
Chapter 5	
Growth-Regulating Compound Conditioning and Priming Improve Establishment and Biomass Quality in Seed-Derived Miscanthus.	137
Chapter 6	
Limits of Growth Manipulation in Early Miscanthus Development: Evaluating Synergy and Priming Effects.	184
Chapter 7	
General Discussion, Conclusions and Recommendations	209

Lay summary

This thesis examines how the early development of *Miscanthus*, a perennial grass widely promoted for bioenergy, can be improved to support net-zero climate targets. *Miscanthus* has attracted significant attention because it produces large amounts of biomass, requires relatively low inputs, and can grow on land that is less suitable for food crops. These characteristics make it a strong candidate for sustainable energy production and carbon mitigation. However, despite this potential, large-scale deployment remains limited. A central reason for this gap between potential and practice is the difficulty of establishing the crop successfully in the field, particularly during the earliest stages of growth.

The research begins from the observation that most existing studies and models focus on mature biomass yield, while underestimating the importance of early development. In perennial systems such as *Miscanthus*, early growth is not a temporary phase that can be corrected later; instead, it sets the trajectory for the entire lifespan of the crop. Poor establishment can lead to reduced plant density, uneven stands, and delayed or reduced yields for many years. In contrast, strong early development increases the likelihood of achieving stable and productive systems. This thesis therefore reframes establishment as a critical developmental stage, arguing that the success of *Miscanthus* depends less on its theoretical yield potential and more on its ability to establish reliably under real-world conditions.

To explore how early development might be improved, the thesis investigates the role of biological interventions, including plant growth regulators (PGR) and biostimulants (BS). These inputs are designed to influence plant growth, for example by affecting internal plant signals, improving stress tolerance, or enhancing nutrient uptake. A review of existing research shows that while these approaches are widely studied, the evidence base is fragmented. Most studies are carried out under controlled conditions, focus on short-term outcomes, and rely on planting methods that are not representative of current large-scale practices. As a result, they provide limited insight into how these treatments perform in real field environments.

The experimental work in this thesis addresses some of these limitations by focusing on seed-derived, plug-based systems, which are increasingly important for large-scale planting. The results show that early development can be influenced. Some PGR and BS treatments improved early plant height, biomass accumulation, and aspects of photosynthetic performance during establishment. This confirms that young *Miscanthus* plants are responsive during this period, and that it is possible to guide their development to some extent.

However, the results also show clear limits to this influence. Improvements observed under controlled conditions often became weaker or disappeared after plants were transferred to field environments. Factors such as soil quality, water availability, and temperature may have had a stronger effect on plant performance than the treatments applied earlier. In addition, early improvements were rarely maintained over time. Gains in growth or plant function during the early stage did not consistently translate into better performance across a full growing season.

The research also revealed that responses to these treatments were often variable. A treatment that worked well in one situation or environment could have little or no effect in another, and sometimes even reduced performance. These differences are not simply experimental inconsistencies but reflect the complexity of plant systems. The effectiveness of PGR and BS depends on several interacting factors, including plant type, environmental conditions, and application rate. Some treatments were only effective within a narrow range, meaning that careful and precise use is essential.

These findings have important implications for the role of *Miscanthus* in net-zero strategies. For the crop to contribute meaningfully to climate mitigation, it must be grown successfully at scale, often on land with less favourable conditions. This requires moving beyond short-term controlled experiments and focusing on long-term, field-based approaches that reflect real growing environments. It also highlights the importance of seed- and plug-based systems, which are more suitable for large-scale deployment but present additional challenges during early growth.

From a practical perspective, PGR and BS should be used carefully and strategically. They are most effective during the early stages of growth, particularly in controlled plug

production systems. However, they should not be viewed as solutions that can overcome poor site conditions or guarantee success. Instead, they should be used alongside other management practices, including appropriate plant selection, soil preparation, and site-specific management.

Overall, this thesis shifts the focus of *Miscanthus* research from maximising yield in mature plants to understanding the importance of early development. It shows that while biological interventions can influence early growth, their effects are limited, context dependent, and often short-lived. Recognising these constraints provides a more realistic foundation for improving *Miscanthus* establishment and supports the development of more reliable and scalable bioenergy systems.

Chapter 1

General Introduction



Bioenergy, net-zero targets, and the role of lignocellulosic crops

The decarbonisation of energy systems is now framed not as a long-term goal but as a near-term constraint on policy, infrastructure, and land use. Integrated assessment models and national climate strategies agree that limiting warming to well below 2 °C requires rapid reductions in fossil fuel use, large-scale deployment of low-carbon electricity, and the development of negative-emissions options capable of delivering durable carbon removal (Butnar *et al.*, 2019 ; IPCC, 2023). Within this framework, bioenergy occupies a structurally ambiguous position. On the one hand, bioenergy with carbon capture and storage (BECCS) is repeatedly embedded in mitigation pathways as a cost-effective route to net-negative emissions, particularly through its proposed capacity to offset residual emissions from hard-to-abate sectors (like transport) while simultaneously generating energy; on the other, its large-scale implementation is contested because of land-use competition, water demand, and potential conflicts with biodiversity and food security (Hastings *et al.*, 2009; Searchinger *et al.*, 2015). The tension between modelled reliance on bioenergy and the biophysical and social constraints on its expansion is now one of the central unresolved issues in net-zero planning. Consequently, the feasibility of many net-zero pathways increasingly depends not only on technological advances in carbon capture, but also on whether sufficient biomass can be produced sustainably, reliably, and at scale.

This ambiguity reflects a deeper conceptual problem: the viability of bioenergy systems cannot be evaluated solely through carbon accounting or techno-economic optimisation (Creutzig *et al.*, 2015; IPCC, 2023). It depends on whether biomass can be produced, harvested, and supplied at scale under real environmental conditions without triggering unacceptable trade-offs in land use, ecosystem function, or rural livelihoods (Lewandowski *et al.*, 2003; Hastings *et al.*, 2009). In practice, this means that the question is not simply how much biomass can be grown in theory, but under what agronomic, climatic, and socio-economic conditions biomass crops can be established and maintained over decades. The agronomic feasibility of bioenergy crops, particularly on land that is marginal for food production, becomes a first-order determinant of whether BECCS and related systems are credible mitigation options rather than artefacts of modelling assumptions (Figure 1).

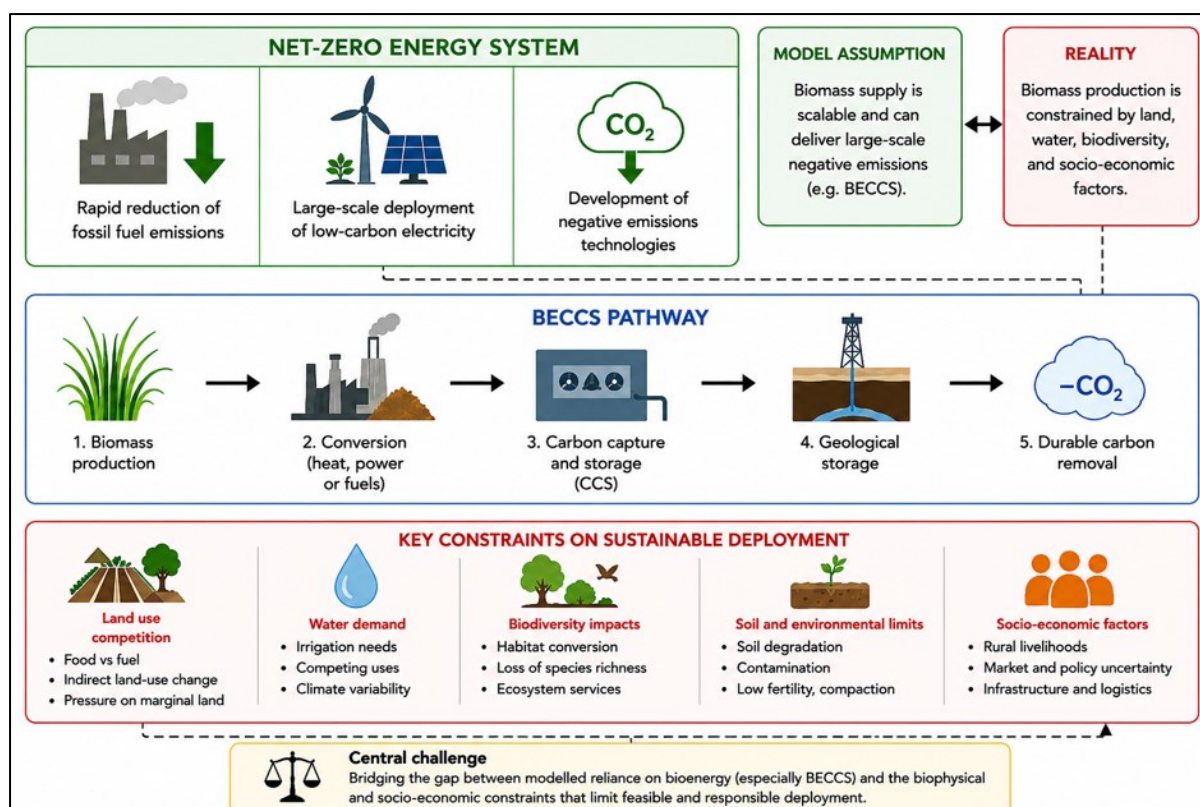


Figure 1. Conceptual representation of the role of biomass within net-zero energy systems, highlighting the integration of bioenergy with carbon capture and storage (BECCS) alongside constraints related to land use, water demand, and biodiversity.

Perennial biomass crops like switchgrass, *Miscanthus*, willow and poplar have emerged as leading candidates within this constrained solution space, typically delivering 7 – 25 t DM ha⁻¹ yr⁻¹ across temperate regions (with global extremes of 3 – 30 t DM ha⁻¹ yr⁻¹) and exhibiting high output:input energy ratios of 20 – 50 (Clifton-Brown *et al.*, 2019). Their perennial growth habit, extensive rooting systems, and relatively low input requirements confer advantages over annual bioenergy crops, especially when deployed on land that is unsuitable or sub-optimal for conventional arable production (Lewandowski *et al.*, 2000; Lewandowski *et al.*, 2016). Among these, *Miscanthus spp.* has been repeatedly identified as a high-potential crop due to its C₄ photosynthetic pathway, a specialised carbon-fixation mechanism that concentrates CO₂ around Rubisco and reduces photorespiration, particularly under high-light and warm conditions, alongside high radiation-use efficiency, and capacity for substantial below-ground carbon allocation (Heaton *et al.*, 2008; Clifton-Brown *et al.*, 2007; McCalmont *et al.*, 2017). Modelling and empirical work suggest that *Miscanthus* can produce high biomass yields while requiring relatively low energy inputs and delivering substantial greenhouse gas emission

reductions when appropriately managed (Hastings *et al.*, 2009; Dohleman *et al.*, 2012; Strullu *et al.*, 2011).

Yet the prominence of *Miscanthus* in scenario analysis and policy discourse stands in marked contrast to its limited real-world deployment (Von Hellfeld *et al.*, 2022; Hodgson *et al.*, 2024). Despite decades of research, demonstration, and policy support through net-zero and biomass expansion strategies in the United Kingdom and Europe, current *Miscanthus* cultivation in the United Kingdom remains estimated at only approximately 8,000 ha. More broadly, the total area of dedicated perennial biomass crops in the UK is estimated at only around 10 000 ha, whereas national net-zero pathways project the need for approximately 700,000 ha by 2050, including establishment rates of at least 30,000 ha yr⁻¹ by 2035 (Von Hellfeld *et al.*, 2022; Hodgson *et al.*, 2024). Current deployment therefore represents only around 1–2% of the perennial biomass area envisaged under UK mitigation scenarios. The scale of this disparity highlights how far bioenergy systems remain from the levels of deployment assumed within net-zero pathways and underscores the difficulty of translating theoretical biomass potential into durable agricultural reality. More fundamentally, it reflects the extent to which projections of bioenergy deployment often depend on assumptions about sustained field performance that may underrepresent the agronomic challenges associated with establishment, early growth, and long-term stand persistence. In many modelling frameworks, *Miscanthus* is represented as a broadly deployable high-yield perennial crop with relatively stable biomass productivity across large spatial scales, whereas in practice its field performance is highly contingent on site conditions, management, genotype, and successful establishment during the first to third years after planting (Hastings *et al.*, 2017; McCalmont *et al.*, 2017; Shepherd *et al.*, 2023). This discrepancy between modelled potential and field-level performance is conceptualised in Figure 2.

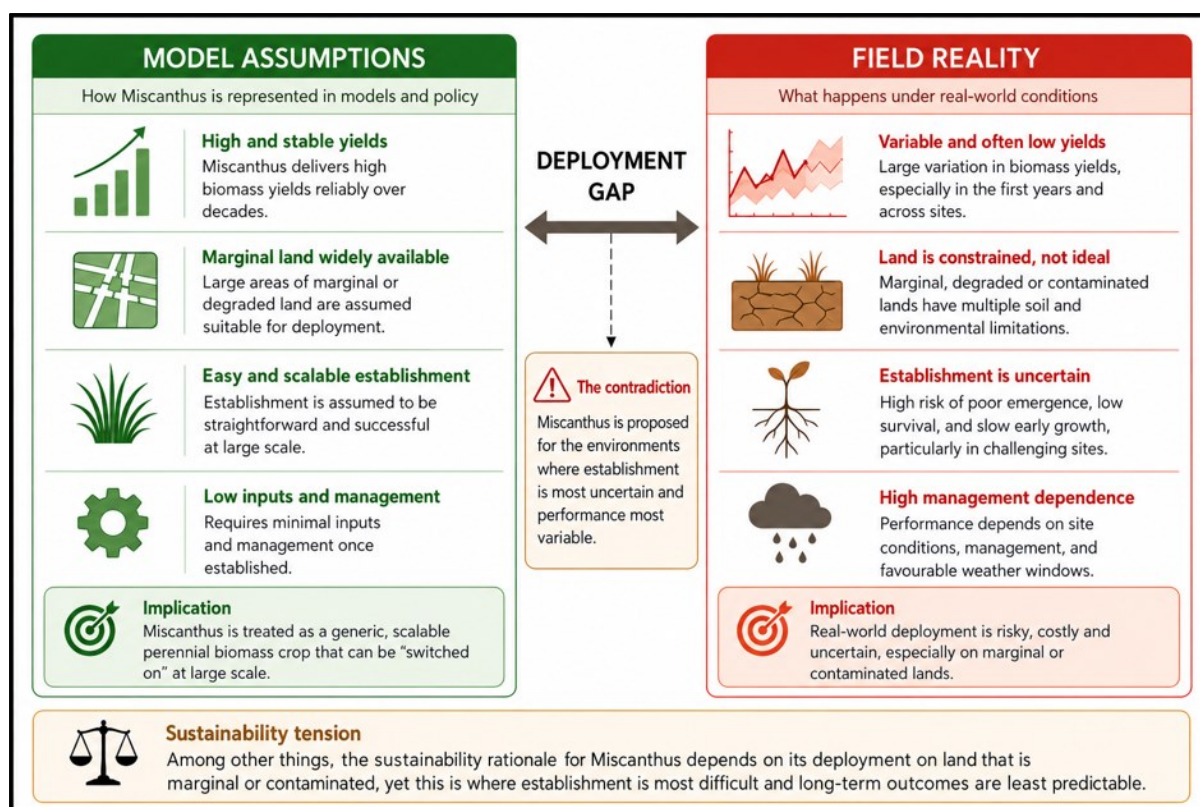


Figure 2. Conceptual illustration of the discrepancy between modelled assumptions of *Miscanthus* deployment and field-level agronomic realities, particularly under marginal land conditions.

The contradiction is sharpened by the spatial logic of sustainable bioenergy. To minimise competition with food crops and reduce indirect land-use change, *Miscanthus* is frequently proposed for marginal, degraded, or contaminated land (Lewandowski *et al.*, 2016; McCalmont *et al.*, 2017). However, these are precisely the environments in which establishment is most difficult, early growth is most variable, and long-term performance is least predictable (McCalmont *et al.*, 2017). The sustainability rationale for *Miscanthus* thus depends on its deployment in contexts that exacerbate, rather than alleviate, agronomic risk (Figure 2). Understanding and manipulating early developmental processes under such constraints becomes central to any credible claim that *Miscanthus* can contribute to net-zero strategies at scale.

Establishment as the primary bottleneck in *Miscanthus* systems

In perennial cropping systems, establishment is not a transient technical hurdle but a determinant of long-term system trajectory. Unlike annual crops, where yield is recalibrated each season, early developmental constraints in perennials propagate across multiple years, shaping stand density, canopy structure, and resource capture

throughout the productive lifespan of the stand (Strullu *et al.*, 2011). For *Miscanthus*, the first two to three growing seasons determine whether a stand develops sufficient canopy cover, rhizome reserves, and plant density to transition into a stable high-yield phase (Clifton-Brown & Lewandowski, 2000; Lewandowski *et al.*, 2000). For example, O’Loughlin *et al.* (2017) reported that peak biomass production is rarely achieved before the third growing season and may take up to five years under suboptimal conditions. Establishment failure or sub-optimal early growth therefore represents not merely an early-stage inefficiency, but a structural limitation on productivity, economic viability, and carbon mitigation potential (Hastings *et al.*, 2017; McCalmont *et al.*, 2017). The long-term implications of establishment success in perennial bioenergy systems are illustrated in Figure 3.

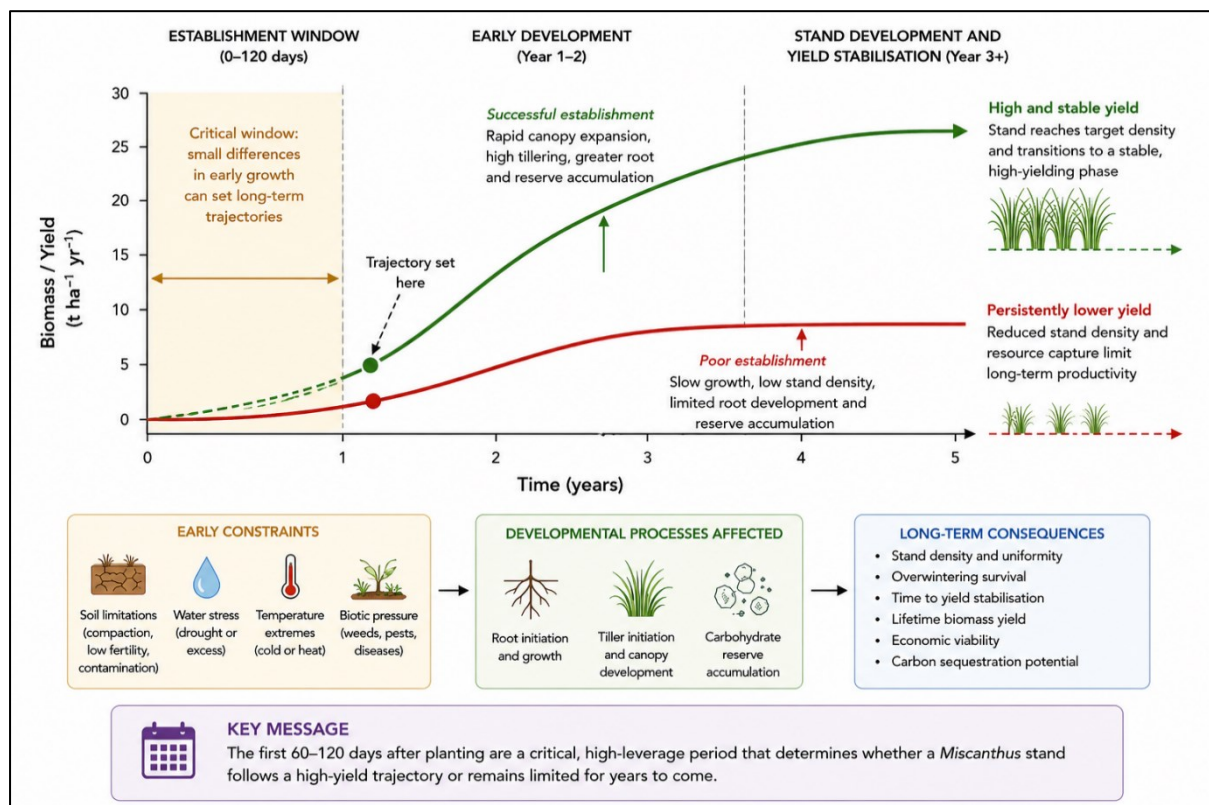


Figure 3. Conceptual representation of the trajectory-setting role of establishment in perennial *Miscanthus* systems, illustrating how early-stage conditions influence long-term yield outcomes.

Despite this, establishment has historically been treated as a secondary consideration relative to yield optimisation in mature stands. Early work on *Miscanthus* focused on genotype evaluation, biomass quality, and combustion properties, often under conditions where establishment was managed intensively or assumed to be non-limiting (Lewandowski *et al.*, 2000; Baxter *et al.*, 2012; Van der Weijde *et al.*, 2017). This

imbalance has contributed to a situation in which *Miscanthus* is well characterised in terms of peak productivity and feedstock quality, but less well understood in terms of the processes that enable that productivity to be realised under field conditions, particularly on marginal land (Figure 3). The agronomic bottleneck is not the physiological capacity of the crop to produce biomass, but the reliability with which that capacity can be expressed across different environments.

Commercial *Miscanthus* cultivation in Europe has historically been dominated by the sterile triploid hybrid *Miscanthus* × *giganteus*, which remains most of the commercially planted stock in the United Kingdom and Europe and is typically propagated vegetatively via rhizomes (Lewandowski *et al.*, 2000; Clifton-Brown *et al.*, 2017). Rhizome-based establishment has the advantage of clonal uniformity and relatively rapid canopy closure under favourable conditions, but it is constrained by high planting costs, logistical complexity, and sensitivity to soil physical properties and moisture availability (Atkinson, 2009; Hastings *et al.*, 2017). Rhizome survival is particularly vulnerable to desiccation, compaction, and poor soil structure; conditions that are characteristic of many marginal or post-industrial sites (Clifton-Brown & Lewandowski, 2000; Lewandowski *et al.*, 2016). The result is a structural contradiction: the sustainability rationale for *Miscanthus* depends on its deployment on marginal land, yet the dominant establishment method performs least reliably under those conditions.

This contradiction is not merely practical but conceptual. It exposes a gap between the way *Miscanthus* is represented in energy system models, as a generic, scalable biomass source, and the way it behaves as a biological system planted in specific soils and climates. It also highlights the importance of early developmental processes as a locus of risk and opportunity. If establishment is the primary bottleneck, then interventions that target early growth, root development, and stress resilience may have disproportionate leverage over long-term outcomes. Conversely, failure to address establishment constraints will limit the scalability of *Miscanthus* regardless of advances in breeding, logistics, or conversion technology (Clifton-Brown *et al.*, 2019; Von Hellfeld *et al.*, 2022).

Shifting propagation pathways and the intensification of early-stage constraints

In response to the limitations of rhizome-based systems, substantial effort has been directed toward the development of alternative propagation pathways, including seed-derived hybrids, plug-based systems, and stem-derived planting material (Clifton-Brown *et al.*, 2017; Lewandowski *et al.*, 2016). Seed-based breeding programmes have expanded the genetic base of *Miscanthus*, enabling the development of inter- and intra-specific hybrids with improved cold tolerance, yield stability, and adaptation to diverse environments (Clifton-Brown *et al.*, 2019; Awty-Carroll *et al.*, 2023). Examples include seed-based *Miscanthus sacchariflorus* × *M. sinensis* hybrids such as OPM-06, GNT43, and GRC14, developed and evaluated through major European projects including OPTIMISC, GIANT-LINK, and GRACE, which aimed to improve establishment success, stress tolerance, yield stability, and adaptation across diverse environments (Clifton-Brown *et al.*, 2017; Clifton-Brown *et al.*, 2019; Awty-Carroll *et al.*, 2023). Seed-derived plug systems have additionally been proposed as a route to reduce establishment costs, improve scalability, and facilitate mechanisation, particularly in large-scale deployment scenarios (Clifton-Brown *et al.*, 2017; Wu *et al.*, 2022; Zheng *et al.*, 2021). The shift in propagation pathways and associated redistribution of establishment constraints is summarised in Figure 4.

Propagation pathway	1. RHIZOME (vegetative)	2. SEED (direct sown)	3. PLUG (seed-raised)	4. STEM (cuttings)
				
 Relative cost (to establish)	High Expensive rhizome production & handling	Low Low seed cost	Medium Nursery inputs (substrate, facilities, labour)	Medium-Low Low material cost, variable rooting success
 Scalability potential	Low-Medium Limited by rhizome supply and logistics	High High potential for large-scale sowing	High Mechanisable transplanting in large areas	Medium Depends on rooting efficiency and propagation rates
 Early-stage robustness	Medium Good reserves, but sensitive to site conditions	Low Tiny seedlings, limited reserves and roots	Medium-High Established seedlings with better root systems	Medium Depends on rooting success and moisture
 Key vulnerabilities	<ul style="list-style-type: none"> Desiccation Poor soil structure High planting cost 	<ul style="list-style-type: none"> Germination failure Drought, crusting, weeds High climatic sensitivity 	<ul style="list-style-type: none"> Transplant shock Handling damage Production cost 	<ul style="list-style-type: none"> Low rooting success Moisture stress Disease risk
 Establishment speed	Fast Rapid shoot emergence and canopy closure	Slow Weeks to months to establish seedlings	Medium-Fast Established plants at transplanting	Variable Depends on rooting and early growth
 Stand uniformity (potential)	High Clonal, uniform planting material	Low-Medium Genetic and emergence variability	High Nursery control promotes uniform stands	Medium Variability in rooting and early growth



COMMON REALITY
All pathways face high vulnerability during early development, particularly in marginal and stressed environments.

SHIFT IN PROPAGATION STRATEGY
From rhizome systems → seed/plug/stem systems

KEY MESSAGE
Alternative propagation routes aim to improve scalability and reduce costs, but redistribute and intensify early-stage establishment constraints.



SHIFT FROM RHIZOME → SEED/PLUG/STEM SYSTEMS INCREASES EARLY-STAGE VULNERABILITY

Figure 4. Comparison of *Miscanthus* propagation pathways, demonstrating how transitions from rhizome-based to seed- and plug-based systems redistribute and intensify early-stage establishment constraints.

However, these innovations do not eliminate establishment constraints; they redistribute and, in some respects, intensify them. Direct-sown seed systems reduce the need for rhizome production and handling but introduce new vulnerabilities associated with germination, seedling emergence, and early growth under field conditions (Ashman *et al.*, 2018). Work on *Miscanthus sinensis* has shown that germination and survival of direct-sown seed are highly sensitive to sowing date, soil temperature, moisture, and the use of mulch films is required, with substantial year-to-year variability (Ashman *et al.*, 2018). Seedlings possess limited root systems and carbohydrate reserves, making them particularly susceptible to drought, soil crusting, and competition from weeds during the first weeks after sowing (Lewandowski *et al.*, 2016). The biological variability inherent in seed populations further complicates stand uniformity and density.

Plug-based systems, in which seedlings are raised under controlled conditions and transplanted as juvenile plants (Wu *et al.*, 2022; Ashman *et al.*, 2023), mitigate some of these risks by buffering early development from field stresses. Recent work has demonstrated that plug size, seedling age, and sowing date strongly influence field establishment and first-year yield in seed-propagated *Miscanthus*, with larger, older

plugs generally conferring greater robustness at transplanting (Ashman *et al.*, 2023; Wu *et al.*, 2022; Zheng *et al.*, 2021). However, plug production introduces its own constraints, including the need for greenhouse infrastructure, substrate, heating, and labour, as well as the risk of transplant shock when plugs are moved into harsher field environments (Ashman *et al.*, 2023). Stem-derived propagation, including the use of stem cuttings, offers another route to clonal multiplication but remains sensitive to rooting success and moisture management (Liu & Ludewig, 2020).

More fundamentally, the shift from rhizome-based to seed- and plug-based systems exposes the intrinsic fragility of early development in *Miscanthus*. Regardless of propagation pathway, young plants are generally characterised by limited root volume, restricted nutrient acquisition capacity, and reduced buffering against environmental stress (Poorter *et al.*, 2016). These constraints are particularly relevant to *Miscanthus* seedlings and juvenile plugs, whose establishment depends on the rapid development of root systems and sufficient resource acquisition to support early growth under field conditions. Their success is contingent on a narrow window of favourable conditions, which is rarely guaranteed in field environments, particularly on marginal or contaminated soils (Lewandowski *et al.*, 2016). The transition to seed-based systems therefore intensifies, rather than alleviates, the importance of early developmental processes as a control point in *Miscanthus* production systems.

This intensification has two implications. First, it increases the premium on management practices and interventions that can stabilise early growth under variable conditions, including sowing date optimisation, mulch use, and plug production protocols (Ashman *et al.*, 2018; Wu *et al.*, 2022; Ashman *et al.*, 2023). Although seed- and plug-based systems can reduce establishment costs relative to conventional rhizome propagation, with some studies reporting reductions from approximately £1,500 ha⁻¹ to below £900 ha⁻¹, these economic benefits depend on achieving high establishment success and can be rapidly eroded by poor emergence, transplant losses, or the need for replanting (Hastings *et al.*, 2017; Clifton-Brown *et al.*, 2017). Second, it creates a conceptual opening for biological interventions, such as plant growth regulators (PGR), biostimulants, and microbial inoculants, that target the physiological and developmental processes underpinning establishment. If early development is both fragile and plastic,

then manipulating hormonal signalling, root architecture, and stress responses during this window may offer a route to reconcile the agronomic demands of large-scale deployment with the environmental constraints of marginal land.

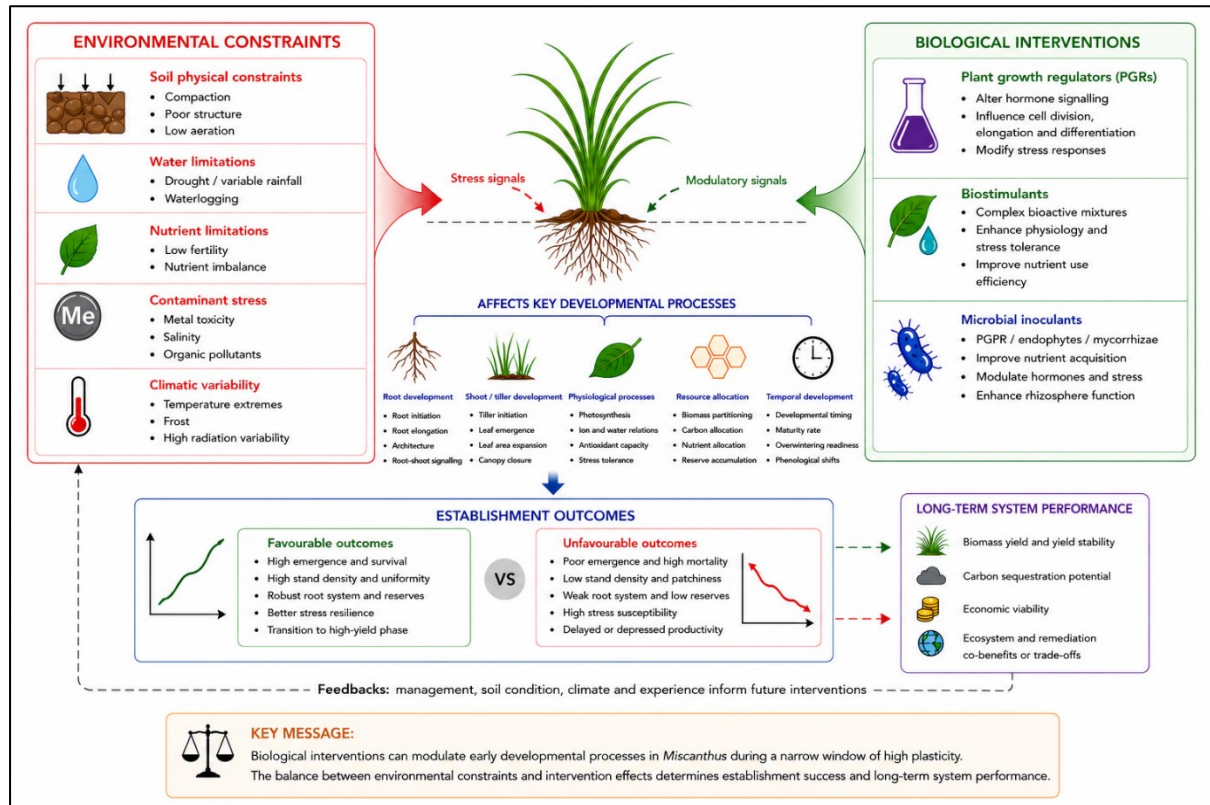


Figure 5. Conceptual framework illustrating early-stage developmental manipulability in *Miscanthus*, showing how environmental constraints and biological interventions interact to influence establishment outcomes and long-term system performance.

Early-stage developmental biology under environmental constraint

Early development in *Miscanthus* involves a coordinated sequence of processes, including root initiation and elongation, tiller formation, canopy expansion, and the accumulation of carbohydrate reserves for overwintering. These processes are regulated by endogenous hormonal networks, principally auxins, cytokinins, gibberellins, abscisic acid, and ethylene, and are highly sensitive to environmental conditions (Figure 5) such as temperature, water availability, nutrient supply, and light (Davies, 2010; Santner & Estelle, 2009). In grasses, the balance between shoot and root growth, the timing of tiller initiation, and the allocation of assimilates to structural versus storage pools are all modulated by hormonal cross-talk and resource availability, with consequences for both short-term growth and long-term stand structure (Schnyder & Nelson, 1988; Wilson, 1988; Poorter *et al.*, 2012).

Under marginal and contaminated conditions, these developmental processes are disrupted by multiple, interacting stressors. Soil compaction restricts root penetration and reduces aeration, limiting access to water and nutrients and altering root–shoot signalling (Lewandowski *et al.*, 2016; Poorter *et al.*, 2016). Nutrient limitations constrain growth and can shift biomass allocation patterns, often favouring root investment at the expense of shoot expansion, with implications for canopy closure and weed suppression (Brouwer, 1962; Shipley & Meziane, 2002; Poorter *et al.*, 2012). In *Miscanthus* specifically, establishment and biomass productivity have been shown to decline substantially under abiotic stress conditions. For example, drought and poor establishment conditions have been associated with reductions in early biomass accumulation and delayed canopy development, while salinity and metal contamination have been reported to impair shoot growth, photosynthetic efficiency, and tiller production (Pidlisnyuk *et al.*, 2020; Malinská *et al.*, 2020; Nurzhanova *et al.*, 2023). Salinity stress has been shown to reduce *Miscanthus* biomass production by more than 50 % under severe conditions in some controlled studies, whereas contaminated soils can significantly suppress establishment success and below-ground development depending on contaminant type and concentration (Nurzhanova *et al.*, 2023; Pidlisnyuk *et al.*, 2020). These constraints rarely occur in isolation; rather, they co-occur and interact, producing a system in which early development is both more difficult to achieve and more consequential for long-term performance. However, despite increasing interest in marginal-land bioenergy systems, important uncertainties remain regarding how multiple stressors interact during the establishment phase and whether early developmental limitations can be mitigated sufficiently to sustain long-term productivity under field conditions (Figure 5).

The temporal dimension of early development is critical. The establishment window, typically encompassing the first few weeks to months after planting, is widely recognised as a phase of heightened vulnerability and developmental plasticity, during which growth trajectories are particularly sensitive to environmental and physiological perturbation (Depuydt & Hardtke, 2011; Poorter *et al.*, 2012; Poorter *et al.*, 2016). In *Miscanthus*, this period generally corresponds to the first 60–120 days after planting, during which root initiation, tiller formation, and early patterns of biomass allocation are established, with consequences for subsequent stand development and yield potential. During this phase,

relatively small perturbations in hormonal signalling, root architecture, or physiological regulation can have disproportionate effects on growth trajectories (Figure 5) and resource capture (Teale *et al.*, 2006; Depuydt & Hardtke, 2011; Vanstraelen & Benková, 2012). For example, auxin and cytokinin gradients regulate the initiation and outgrowth of tillers and buds, influencing canopy architecture and competitive ability (Domagalska & Leyser, 2011; Müller & Leyser, 2015). Gibberellins modulate stem elongation and leaf expansion, with well-characterized effects on lodging and biomass partitioning in grasses (Peng *et al.*, 1999), while abscisic acid plays a central role in drought responses and stomatal regulation (Verma *et al.*, 2016).

Existing *Miscanthus* research has focused predominantly on biomass yield, environmental adaptation, genotype performance, and agronomic management across contrasting climatic and edaphic conditions (Lewandowski *et al.*, 2000; Clifton-Brown *et al.*, 2004; Hastings *et al.*, 2009; Heaton *et al.*, 2010), whereas comparatively fewer studies have examined the mechanistic regulation of early developmental processes or the role of plant growth regulators during establishment under marginal conditions (Chapter 2). Nevertheless, genotypic differences in cold tolerance, stay-green behaviour, and growth rate have been linked to variation in developmental and physiological responses, with important consequences for overwintering and yield stability (Clifton-Brown & Lewandowski, 2000; Clifton-Brown *et al.*, 2004; Robson *et al.*, 2013).

From a systems perspective, early development can be viewed as a phase in which the plant is setting its trajectory within a given environment. The balance between shoot and root growth, the timing of tiller initiation, and the accumulation of below-ground reserves all influence whether a stand will achieve target density, survive winter, and enter a high-yield phase within the expected timeframe (Strullu *et al.*, 2011; Dohleman *et al.*, 2012). Environmental constraints during this phase can push the system towards alternative trajectories (Figure 3), including low-density stands, patchy establishment, or repeated replanting, thereby undermining both economic and environmental performance. Conversely, interventions that stabilise or enhance early development under stress, including improved planting material, targeted nutrient management, seed or rhizome priming, and the application of plant growth regulators or biostimulants, may generate

cascading benefits over the lifespan of the crop by improving establishment success, stand uniformity, stress tolerance, and long-term productivity.

Biological interventions: potential and limitation

Plant growth regulators (PGR), biostimulants (BS), and microbial inoculants (MBI) have been proposed as tools to exploit this (establishment) window of developmental plasticity (Obaje *et al.*, 2026). Conceptually, these interventions offer a means to manipulate early growth processes in ways that enhance establishment success, stress resilience, and, in some contexts, contaminant uptake or stabilisation. They operate at different levels of the plant–soil–microbe system: PGR act directly on hormonal signalling pathways; BS modulate physiological and biochemical processes through complex mixtures of bioactive compounds; and MBI reshape rhizosphere interactions and nutrient dynamics (Davies, 2010; du Jardin, 2015; Calvo *et al.*, 2014; Kaushal *et al.*, 2023).

Plant growth regulators, whether natural or synthetic, act at low concentrations on hormone receptors and signalling cascades that govern cell division, elongation, organogenesis, and stress responses (Davies, 2010; Rademacher, 2015). In annual crops, exogenous application of auxins, gibberellins, cytokinins, and growth retardants has been used to manipulate root development, stem elongation, and reproductive allocation, with well-documented effects on lodging resistance and yield (Peng *et al.*, 1999; Rademacher, 2015; Severino & Oliveira, 2024). In *Miscanthus*, studies conducted under contaminated and nutrient-poor conditions have shown that specific PGR and biostimulant-associated treatments can alter biomass accumulation, physiological performance, and metal uptake dynamics. For example, Pidlisnyuk *et al.* (2022) reported that the PGR Charkor enhanced the development and dry biomass accumulation of *Miscanthus × giganteus* grown on marginal post-military soils, with effects becoming more pronounced during the second year of vegetation. Similarly, Malinská *et al.* (2020) showed that treatments with Stimpo and Regoplant modified biomass parameters and metal uptake responses, although effects varied depending on soil condition and treatment combination. The same hormonal pathways that confer potential leverage also introduce complexity: PGR effects are strongly modulated by developmental stage, environmental conditions, and interactions with endogenous hormone pools (Santner & Estelle, 2009; Depuydt & Hardtke, 2011). This suggests that hormonal manipulation may

offer a means of improving establishment under environmentally constrained conditions, but also highlights the challenge of achieving consistent responses across deployment contexts.

Biostimulants constitute a broader and more heterogeneous category, encompassing humic substances, protein hydrolysates, seaweed extracts, microbial consortia, and other formulations that enhance plant performance through mechanisms other than direct nutrient supply (du Jardin, 2015; Rouphael & Colla, 2020; Calvo *et al.*, 2014). Their reported effects include improved nutrient uptake, enhanced photosynthetic efficiency, modulation of antioxidant systems, and increased tolerance to abiotic stress (Cristofano *et al.*, 2021; Urbutis *et al.*, 2023). In *Miscanthus*, biostimulant applications have been less extensively studied than in horticultural crops, but emerging work suggests potential benefits for biomass yield and stress resilience, particularly when combined with microbial inoculants on marginal or contaminated soils (Fei *et al.*, 2020; Peroni *et al.*, 2024; Iqbal *et al.*, 2025). For example, Peroni *et al.* (2024) reported that biostimulant-assisted treatments improved physiological performance and biomass responses of *Miscanthus* cultivated under environmentally constrained conditions, supporting their potential role in enhancing establishment and stress adaptation. However, the complexity of biostimulant formulations and the context dependence of their effects limit predictive understanding and complicate optimisation. These characteristics are particularly relevant to seed- and plug-based *Miscanthus* systems, where small improvements in early root development, stress tolerance, or nutrient acquisition may have disproportionate effects on establishment success.

Microbial inoculants extend the intervention space by targeting the rhizosphere and endosphere. Plant growth-promoting bacteria and mycorrhizal fungi can enhance nutrient acquisition, modulate hormone levels, and improve tolerance to abiotic stress, including salinity and metal toxicity (Backer *et al.*, 2018; Kaushal *et al.*, 2023). In *Miscanthus*, endophytic and rhizosphere bacteria have been shown to promote growth, increase metal uptake, and enhance tolerance on contaminated soils, often through combined effects on nutrient mobilisation and hormonal signalling (Straub *et al.*, 2013; Liu & Ludewig, 2020; Liu *et al.*, 2021; Xiao *et al.*, 2022; Wang *et al.*, 2023; Wang *et al.*, 2025). For example, Straub *et al.* (2013) demonstrated that inoculation with the bacterial

endophyte *Herbaspirillum frisingense* enhanced growth of *Miscanthus sinensis* through modulation of root development and ethylene signalling. Similarly, Liu *et al.* (2021) reported that inoculation of *Miscanthus floridulus* with the plant growth-promoting bacterium *Klebsiella michiganensis* TS8 increased plant height by 39.9% and leaf dry biomass by 99.1% under cadmium-contaminated conditions, while also reducing rhizosphere Cd concentrations. In the same study, *Lelliottia jeotgali* MR2 enhanced cadmium translocation to aboveground tissues, highlighting the capacity of microbial inoculants to influence both biomass production and phytoremediation performance.

Arbuscular mycorrhizal fungi and dark septate endophytes have similarly been implicated in improved growth and metal tolerance in *Miscanthus sacchariflorus* and *M. sinensis* on mine soils (Sarkar *et al.*, 2018; Haruma *et al.*, 2021; Lu *et al.*, 2024). For instance, Haruma *et al.* (2021) found that inoculation with dark septate endophytes improved plant growth and metal tolerance in *Miscanthus sinensis* growing on post-mining substrates. Yet, as with PGR and biostimulants, the performance of microbial inoculants is contingent on complex interactions with native microbial communities, soil properties, and plant genotype, leading to variable outcomes between controlled environments and field conditions.

Taken together, these interventions represent high-potential but low-predictability tools. Their effects are inherently context-dependent, shaped by the intersection of plant developmental stage, environmental stressors, and background soil biology. Optimising their use in *Miscanthus* establishment therefore requires a more mechanistic and system-level understanding than is currently available. It also demands careful consideration of trade-offs, particularly in phytomanagement systems where biomass production, contaminant dynamics, and ecosystem services must be balanced (Pidlisnyuk *et al.*, 2020; Ofori-Agyemang *et al.*, 2024; Peroni *et al.*, 2025).

Trade-offs and multi-functional constraints

The complexity of establishment interventions is further amplified in phytomanagement contexts, where *Miscanthus* is expected to deliver multiple functions, including biomass production, carbon sequestration, and soil remediation. On metal-contaminated or post-industrial sites, *Miscanthus* is often deployed as part of integrated strategies that

aim to stabilise or extract contaminants while producing biomass for energy or bioproducts (McCalmont *et al.*, 2017; Pidlisnyuk *et al.*, 2020; Shavyrkina *et al.*, 2023). In such systems, interventions cannot be evaluated solely in terms of biomass. For example, increased growth may dilute contaminant concentrations in plant tissue, reducing phytoextraction efficiency, while enhanced metal uptake may impose physiological costs that reduce biomass production or compromise feedstock quality (Nebeská *et al.*, 2019; Pidlisnyuk *et al.*, 2019; Lutts *et al.*, 2025).

These trade-offs are not incidental; they reflect fundamental constraints in how plants allocate resources under stress and how contaminants move through plant–soil systems. Enhanced root growth and rhizosphere activity may improve both nutrient and metal uptake, but the relative partitioning of metals between roots and shoots determines whether a system functions primarily as a stabilisation or extraction technology (Babu *et al.*, 2015; Liu *et al.*, 2021; Xiao *et al.*, 2024). PGR and BS that stimulate growth or alter transpiration can shift these balances in ways that are beneficial or detrimental depending on the management objective (Malinská *et al.*, 2020; Pidlisnyuk *et al.*, 2022; Peroni *et al.*, 2024). MBI may enhance microbial diversity and soil function, but their effects on contaminant speciation and mobility can be complex and site-specific (Nurzhanova *et al.*, 2023; Iqbal *et al.*, 2025).

A key limitation of existing research is its tendency to evaluate these outcomes in isolation, rather than as components of an integrated system. Many studies focus on growth responses without detailed assessment of physiological or remediation metrics, or vice versa (Fei *et al.*, 2020; Schmidt *et al.*, 2017; Pidlisnyuk *et al.*, 2022). This fragmentation obscures the trade-offs that are central to deployment decisions. For bioenergy-oriented systems, the primary concern may be yield stability and combustion quality, whereas for phytoremediation the priority may be contaminant removal or immobilisation, even at the cost of reduced biomass. In practice, many projects occupy an intermediate position, seeking to combine biomass production with risk reduction, which makes the explicit characterisation of trade-offs essential (McCalmont *et al.*, 2017; Ofori-Agyemang *et al.*, 2024; Peroni *et al.*, 2025).

Gaps and biases in the *Miscanthus* intervention literature

The limitations outlined above are reinforced by structural biases in the existing literature on *Miscanthus* and biological interventions. As demonstrated in the scoping review presented in Chapter 2, the evidence base is characterised by a narrow range of experimental systems, with a strong emphasis on *Miscanthus* × *giganteus*, rhizome-based propagation, and controlled or semi-controlled environments. Seed- and plug-based systems, which are central to current deployment strategies and breeding programmes, are comparatively under-represented, particularly in field contexts (Clifton-Brown *et al.*, 2017; Lewandowski *et al.*, 2016; Ashman *et al.*, 2018; Wu *et al.*, 2022).

Short-term experiments dominate the literature, often concluding within a single growing season or even within a few weeks or months. These studies are valuable for elucidating acute physiological responses and mechanistic pathways, but they are poorly aligned with the perennial nature of *Miscanthus* and the multi-year timescales over which establishment, overwintering, and yield stabilisation occur (Hastings *et al.*, 2017; McCalmont *et al.*, 2017). Growth metrics, such as plant height, leaf area, and above-ground biomass, are prioritised, while physiological indicators (e.g. chlorophyll fluorescence, gas exchange, antioxidant activity) and long-term performance metrics (e.g. stand density, yield trajectory, carbon balance) are less consistently assessed. Critically, integrated assessments that simultaneously consider growth, physiology, and remediation outcomes remain rare.

This has resulted in a situation where mechanistic understanding is often greatest for experimental systems that are less representative of current deployment pathways, whereas evidence from deployment-relevant systems remains comparatively limited. Controlled environment studies on rhizome-propagated *Miscanthus* × *giganteus* provide detailed insights into specific mechanisms but offer limited guidance for seed-based plug systems on marginal soils. Conversely, field trials that are directly relevant to deployment often lack the physiological and mechanistic depth needed to interpret variable responses to interventions (Lewandowski *et al.*, 2016; Fei *et al.*, 2020; Pidlisnyuk

et al., 2022). Such an imbalance constrains both synthesis and application and contributes to the persistent gap between experimental outcomes and field performance.

Chapter 2 addresses these issues by systematically mapping the distribution of studies across species, planting materials, environments, soil contexts, intervention classes, and outcome domains. It identifies where experimental choices cluster, where evidence is sparse or contradictory, and how reported effects are distributed across growth, physiological, and remediation domains. This mapping provides the empirical foundation for the experimental work in subsequent chapters, which explicitly targets under-represented combinations of propagation pathway, intervention type, and environmental context.

Thesis rationale and structure

This thesis proceeds from the premise that early-stage developmental processes represent the primary, yet systematically under-optimised, control point in *Miscanthus* production systems. The central question is not whether *Miscanthus* can produce high yields under ideal conditions, this is already well established (Heaton *et al.*, 2008; Clifton-Brown *et al.*, 2007; Van der Weijde *et al.*, 2017), but whether its early development can be manipulated in ways that are robust to environmental variability and scalable across deployment contexts, including marginal and contaminated soils. Biological interventions in the form of plant growth regulators, biostimulants, and microbial inoculants offer a promising but poorly resolved toolkit for this purpose.

Chapter 2 provides a scoping review and evidence map of peer-reviewed studies on plant growth regulators, biostimulants, and growth-promoting microorganisms in *Miscanthus* from 2010 to 2025, following PRISMA-ScR (Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews) guidelines. It characterises the structural biases of the literature, the distribution of outcome domains across intervention classes, and the direction of reported effects, thereby identifying critical knowledge gaps and methodological limitations. This chapter establishes the broader context within which the experimental chapters are positioned.

Chapters 3–5 present targeted experimental work that interrogates the effects of biological interventions on early growth, physiology, and biomass allocation in *Miscanthus* under controlled and field conditions. These chapters focus on seed- and plug-based systems, explicitly addressing the propagation pathways that are most relevant to scalable deployment but least represented in the existing evidence base (Clifton-Brown *et al.*, 2017; Ashman *et al.*, 2018; Wu *et al.*, 2022). They integrate growth measurements with physiological indicators such as chlorophyll fluorescence, and pigment composition, enabling a more mechanistic interpretation of treatment effects (Baker, 2008; Ruban, 2016; Shimakawa & Miyake, 2018).

Chapter 6 reports a set of results that diverged from expected patterns, highlighting the limits of current conceptual models and the context-dependence of intervention outcomes. Rather than treating these findings as anomalies, the chapter uses them to interrogate underlying assumptions about developmental plasticity, resource allocation, and stress responses in *Miscanthus*. This reflexive approach is essential for developing conceptually rigorous conclusions in a field where variability and contingency are intrinsic features rather than experimental noise (Des Marais *et al.*, 2013; Poorter *et al.*, 2016).

Chapter 7 synthesises the evidence from the scoping review and experimental chapters to develop a more integrated understanding of establishment optimisation in *Miscanthus*. It articulates a conceptual framework in which early developmental manipulability is positioned as a node linking biological interventions, establishment outcomes, and long-term system performance.

Within this framework, the thesis evaluates when, how, and under what constraints biological interventions (specifically, PGR and BS) can contribute to scalable *Miscanthus* systems that are compatible with net-zero and phytomanagement objectives. The aim is not to advocate uncritically for biological inputs, but to delineate the conditions under which they can be deployed as part of a rational, evidence-based strategy for establishment-phase optimisation.

Aim and Objectives

Aim

The aim of this thesis is to evaluate whether early-stage application of plant growth regulators and biostimulants during plug production can be used to manipulate developmental trajectories in *Miscanthus* in ways that improve establishment performance, and to define the conditions, persistence, and limits of such interventions across controlled and field-relevant systems.

Objectives

1. To map and critically evaluate the existing evidence base on biological interventions in *Miscanthus*

Conduct a PRISMA-ScR scoping review to characterise how studies on plant growth regulators, biostimulants, and related interventions are distributed across species, planting materials, environments, application strategies, and outcome domains, and to identify structural biases and knowledge gaps that constrain inference and inform the design of plug-based experiments (Chapter 2).

2. To quantify early growth and biomass allocation responses to PGR and BS treatments in plug-grown *Miscanthus*

Determine how selected interventions influence early morphological development, biomass accumulation, and root–shoot allocation under controlled, semi-controlled and field conditions, with particular emphasis on variability across treatments and experimental contexts (Chapters 3–5).

3. To characterise physiological responses as indicators of underlying mechanisms

Assess whether PGR and BS treatments alter photosynthetic performance and stress-related processes through measurements of chlorophyll fluorescence, pigment composition, and related physiological indicators, and evaluate the consistency of these responses with proposed mechanisms of action (Chapters 3–6).

4. To test the persistence and transferability of treatment effects beyond the plug stage

Evaluate whether treatment effects observed during early development persist following transplanting and extend to pot and field conditions, including assessment of response stability over at least one growing season (Chapters 4-6).

5. To define application thresholds, variability, and limits of responsiveness

Investigate dose–response relationships and identify thresholds beyond which interventions lose efficacy or produce inconsistent outcomes, and analyse divergent responses to delineate the robustness and limits of early-stage developmental manipulation in *Miscanthus* (Chapter 5 & 6).

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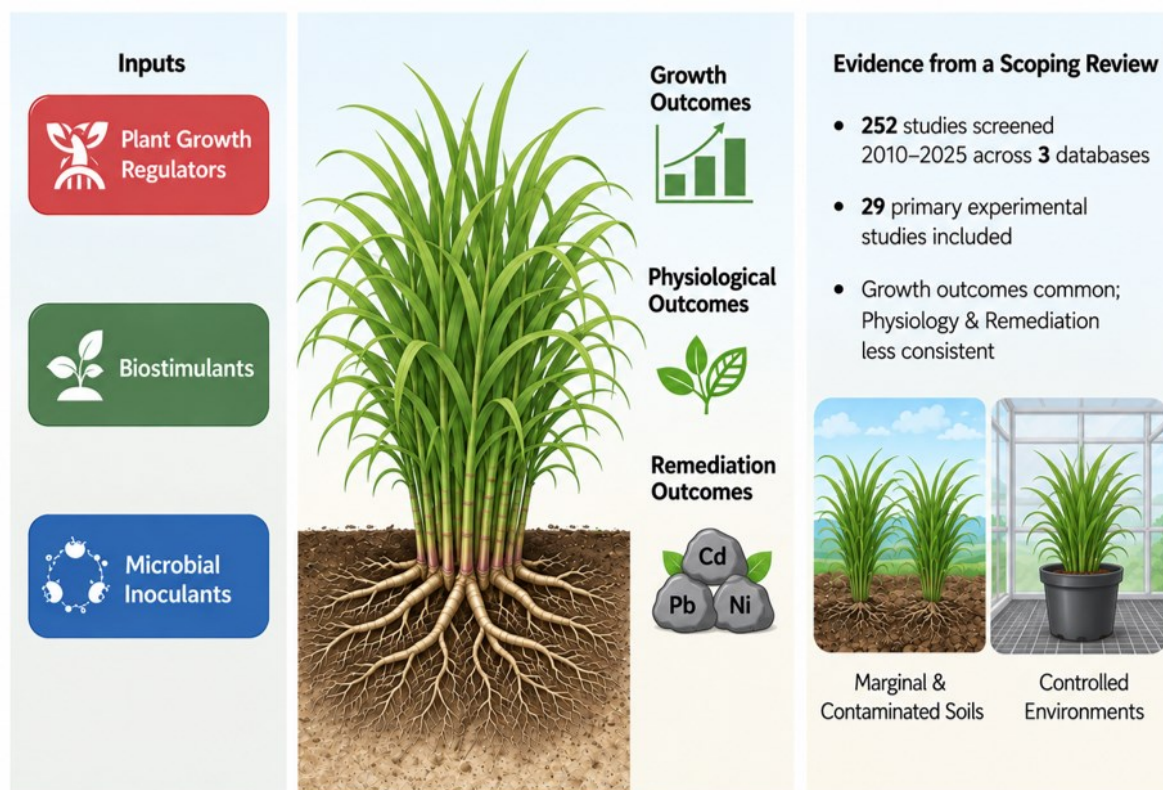
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Chapter 2

Review: Plant Growth Regulators, Biostimulants, and Microbial Inoculants in *Miscanthus*: A Scoping Review of Evidence, Experimental Designs, and Research Gaps (2010–2025).



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ABSTRACT

Miscanthus is a leading lignocellulosic crop for low-carbon bioenergy systems, yet its deployment is limited by establishment challenges, early-stage growth variability, and performance constraints on marginal or contaminated soils. Plant growth regulators (PGR), biostimulants (BS), and growth-promoting microorganisms (GPM) have been proposed to enhance establishment and stress tolerance, but evidence remains fragmented across species, environments, and application strategies. This scoping review systematically mapped peer-reviewed experimental studies published between 2010 and 2025, following PRISMA-ScR guidelines. Searches across Scopus, Web of Science, and PubMed identified 252 records, of which 29 met inclusion criteria after screening and deduplication. Data extraction captured species, planting material, growth environment, soil context, intervention class, application strategy, outcome domains, and effect direction. The evidence base is dominated by *Miscanthus x giganteus*, rhizome-based planting material, controlled environments, and short-duration studies. GPM and BS were the most frequently tested interventions, typically applied early via soil drenching or soaking. Growth outcomes were widely reported and predominantly positive, whereas physiological and remediation outcomes were less consistently assessed and exhibited greater heterogeneity. The review highlights substantial methodological clustering and identifies critical gaps, particularly in long-term field studies, seed- and plug-based systems, and integrated physiological–remediation assessments, needed to support scalable, net-zero-aligned *Miscanthus* deployment.

Keywords: *Miscanthus*, Plant Growth Regulators, Biostimulants, growth-promoting microorganisms, Net-zero, PRISMA-ScR, Bioenergy Crops.

INTRODUCTION

The decarbonisation of energy systems remains central to achieving national and international net-zero targets, with bioenergy expected to play a strategic role alongside electrification and negative emissions technologies (Butnar *et al.*, 2019). Among lignocellulosic energy crops, *Miscanthus* species have emerged as leading candidates due to their high biomass productivity, low input requirements, favourable energy balance, and capacity to grow on marginal or contaminated land without direct competition with food crops (Clifton-Brown *et al.*, 2019; Hastings *et al.*, 2017). As a perennial C₄ grass, *Miscanthus* combines high radiation-use efficiency with long stand lifetimes, enabling substantial carbon sequestration above- and below-ground (McCalmont *et al.*, 2017). These attributes position *Miscanthus* as a cornerstone crop within low-carbon bioenergy and broader circular bioeconomy strategies.

Despite its recognised potential, the large-scale deployment of *Miscanthus* remains constrained by challenges associated with establishment, early growth variability, and site-specific performance, particularly on marginal or contaminated soils (Lewandowski *et al.*, 2016; Hastings *et al.*, 2017). Establishment failure or sub-optimal early growth has disproportionate consequences for perennial crops, as early setbacks can reduce yield for multiple subsequent seasons and compromise economic viability (Ashman *et al.*, 2018). Historically, commercial *Miscanthus x giganteus* has been established through rhizome propagation, a labour-intensive, costly, and sometimes a vulnerable method that results in poor survival under unfavourable conditions (Clifton-Brown *et al.*, 2017). In response, increasing attention has been directed toward alternative establishment pathways, including seed-derived hybrids, plug-based systems, and stem propagation, all of which aim to reduce costs while improving scalability and uniformity (Ashman *et al.*, 2023a; 2023b; Awty-Carroll *et al.*, 2023).

Within this context, manipulating early plant development with plant growth regulators (PGR), biostimulants (BS), and growth-promoting microorganisms (GPM) has attracted growing interest. These inputs are proposed as tools to enhance establishment success, stimulate early biomass accumulation, mitigate abiotic stress, and, in some cases, modulate contaminant uptake in phytomanagement systems (Pidlisnyuk *et al.*, 2020; Peroni *et al.*, 2024). PGR, either natural or synthetic compounds applied at low

concentrations, regulate hormonal signalling pathways that govern plant development, including cell division, elongation, flowering, and stress responses (Davies, 2010). BS, a broader and more heterogeneous category, include humic substances, protein hydrolysates, seaweed extracts, and microbial consortia, which act through indirect physiological and biochemical mechanisms rather than direct nutrient supply (du Jardin, 2015; Rouphael & Colla, 2020). GPM, including plant growth-promoting rhizobacteria, endophytes and mycorrhizal fungi, enhance nutrient acquisition, produce phytohormones, improve root architecture, and increase tolerance to abiotic stress, and are increasingly applied in sustainable agriculture (Backer *et al.*, 2018; Compant *et al.*, 2019).

For *Miscanthus*, these interventions are particularly relevant during establishment phases when plants exhibit limited root systems, reduced nutrient acquisition capacity, and heightened sensitivity to soil and environmental constraints. These include compaction, which can restrict root penetration and water movement; salinity, which imposes osmotic and ionic stress; metal contamination, which can impair root function and induce toxicity; low fertility, which limits nutrient availability and early biomass accumulation; and weed competition, which can restrict access to light, water, and nutrients before a sufficiently developed canopy is established (Malinská *et al.*, 2020; Janczak-Pieniążek *et al.*, 2024). Controlled environment studies have demonstrated that exogenous application of PGR, BS, or GPM can enhance photosynthetic performance, antioxidant capacity, and biomass accumulation under stress conditions (Guo *et al.*, 2017; Sarkar *et al.*, 2018). Field-based experiments further suggest that BS and GPM associations may improve yield stability and support phytoremediation objectives on contaminated or post-industrial land (Pidlisnyuk *et al.*, 2020; Ofori-Agyemang *et al.*, 2024).

However, despite a rapidly expanding body of literature, evidence on the effectiveness of PGR, BS, and GPM in *Miscanthus* remains fragmented. Studies vary widely in species (*Miscanthus x giganteus*, *Miscanthus sinensis*, *Miscanthus sacchariflorus*, *Miscanthus floridulus*), planting material (rhizomes, plugs, seeds, stems), growth environment (growth chambers, greenhouses, field trials), soil context (non-contaminated, marginal, or metal-contaminated), and experimental duration. Moreover, outcomes are reported

across multiple domains, including morphological growth, biomass accumulation, physiological performance, and remediation efficiency, often with divergent or even contradictory results (Schmidt *et al.*, 2017; Pidlisnyuk *et al.*, 2020).

A further complication lies in the temporal scale of existing studies. Many experiments focus on short-term responses spanning weeks to a few months, which may capture transient physiological effects but fail to reflect longer-term consequences for perennial yield formation and stand persistence (Hastings *et al.*, 2017). This limitation is particularly salient for *Miscanthus*, where yield potential and carbon balance are realised over multi-year cycles. While plug-based and seed-derived systems have been promoted as routes to scalable establishment (Ashman *et al.*, 2018; 2023a; 2023b Awty-Carroll *et al.*, 2023), evidence on how early PGR or BS interventions translate into sustained field performance remains sparse.

In parallel, *Miscanthus* has gained prominence within phytomanagement and phytoremediation research, especially on metal-contaminated soils. In such systems, PGR and microbial inoculants are not only evaluated for their effects on biomass production but also for their influence on metal uptake, translocation, and stabilisation (Pidlisnyuk *et al.*, 2020; Nurzhanova *et al.*, 2023). This dual objective introduces additional complexity, as treatments that enhance growth may reduce metal uptake, and vice versa, leading to trade-offs that must be explicitly considered when assessing suitability for clean bioenergy production (Lutts *et al.*, 2025).

To date, no comprehensive synthesis has systematically mapped how PGR, BS, and GPM have been applied in *Miscanthus* research, or addressed where experimental choices cluster across species, environments, and application strategies. Nor has any synthesis thoroughly assessed how reported effects are distributed across growth, physiological, and remediation domains, or highlighted the specific areas where evidence is sparse or contradictory. Previous reviews have addressed *Miscanthus* agronomy more broadly (Lewandowski *et al.*, 2016; Clifton-Brown *et al.*, 2019) or focused on phytoremediation using energy crops (Pidlisnyuk *et al.*, 2022a), but none have explicitly interrogated the evidence base to identify research gaps and methodological limitations relevant to plant growth regulation and establishment optimisation.

Accordingly, the objective of this study is to conduct a scoping review with evidence mapping to systematically characterise peer-reviewed research on the application of plant growth regulators (PGR), biostimulants (BS), and growth-promoting microorganisms (GPM) in *Miscanthus* from 2010 to 2025. Specifically, this review aims to: (i) map the distribution of studies across *Miscanthus* species, planting materials, growth environments, soil contexts, and experimental durations; (ii) categorise application strategies in terms of compound class, frequency, route, and developmental stage; (iii) synthesise reported outcomes across growth, physiological, and remediation domains, including the direction of observed effects; and (iv) identify knowledge gaps and methodological biases that constrain translation to field-scale bioenergy systems.

By consolidating this evidence, the review seeks to inform future experimental design and deployment strategies for *Miscanthus* as a clean energy crop, with particular emphasis on establishment-phase interventions that align productivity gains with net-zero and sustainability objectives.

METHODS

Review design and scope

This study was conducted as a scoping review with evidence mapping, following established methodological guidance for scoping reviews (Arksey and O'Malley, 2005; Levac *et al.*, 2010) and reported in line with the PRISMA-ScR (Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews) framework (Tricco *et al.*, 2018). A scoping approach was selected to capture the breadth, diversity, and methodological heterogeneity of research examining the use of PGR, BS, and GPM in *Miscanthus species*, rather than to quantify pooled effect sizes.

The review focused on peer-reviewed experimental studies published between 2010 and 2025, reflecting the period during which PGR, BS, GPM, and novel establishment strategies have expanded rapidly within bioenergy crop research (Figure S1). The primary objective was to map experimental designs, application strategies, outcome domains, and reported effect directions relevant to *Miscanthus* optimisation for bioenergy and phytomanagement applications.

Literature search strategy

A comprehensive literature search was conducted across Web of Science (WoS Core Collection), Scopus, and PubMed, selected to ensure broad coverage of agronomic, environmental, physiological, and biomedical plant science literature. Searches were restricted to English-language, peer-reviewed research articles.

Search strings were developed iteratively to capture three core conceptual components:

- (i) *Miscanthus* species,
- (ii) plant growth regulators, biostimulants, or growth-promoting microorganisms, and
- (iii) growth, biomass, or physiology-related outcomes.

The final database-specific search strings are reported in full in the Supplementary Material (Table S1) to ensure reproducibility. Briefly, all searches combined *Miscanthus*-related terms with an extensive list of PGR, hormone, biostimulant, and microbial keywords (e.g. auxins, cytokinins, gibberellins, abscisic acid, ethylene, humic substances, biostimulants, PGPR), and outcome-related terms (e.g. growth, biomass, yield, photosynthesis, chlorophyll).

Searches returned the following initial results:

Scopus: 95 records; Web of Science: 111 records; PubMed: 46 records

Study screening and eligibility criteria

Study selection was conducted in three sequential stages: title screening, abstract screening, and full-text eligibility assessment. Title screening reduced the dataset to studies that explicitly involved *Miscanthus* and the experimental application of PGR, BS, or GPM. Abstract screening further excluded studies that did not report plant-level growth, physiological, or remediation outcomes.

Following abstract screening, the number of potentially eligible studies was:

Scopus: 27; Web of Science: 24; PubMed: 16

Cross-database comparison and duplicate removal resulted in a final dataset of 29 unique studies included in the review. A flow diagram summarising the search and screening process is presented in Figure 1.

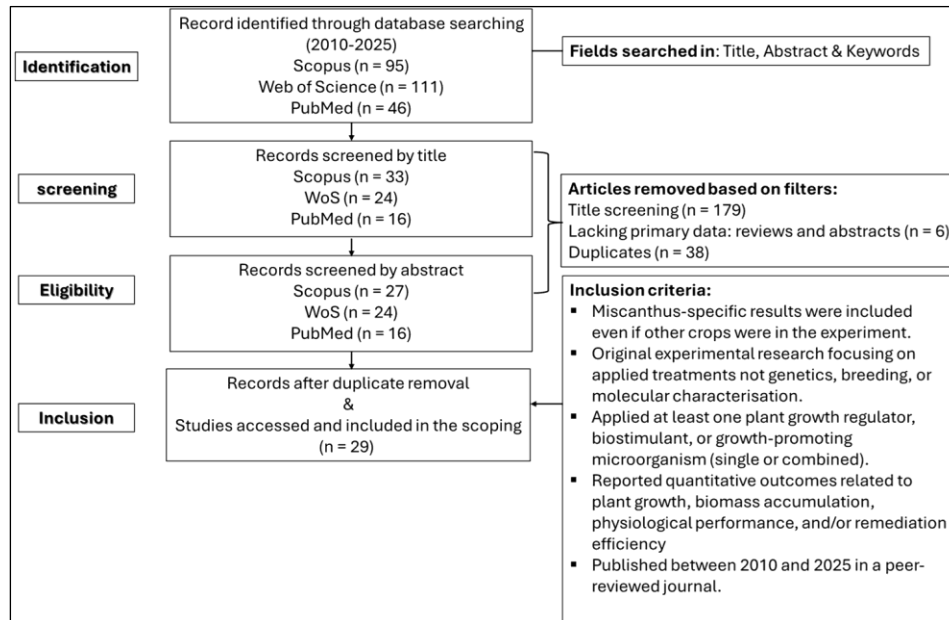


Figure 1. PRISMA-ScR flow diagram of study identification and selection.

Flow diagram illustrating the identification, screening, eligibility assessment, and inclusion of studies in the scoping review. Database searches conducted in Web of Science, Scopus, and PubMed between 2010 and 2025 yielded 252 records. Following title and abstract screening and removal of duplicates, 29 studies met all inclusion criteria and were included in the final evidence synthesis. Search fields included title, abstract, and keywords, and inclusion criteria required experimental application of at least one plant growth regulator, biostimulant, or growth-promoting microorganism to *Miscanthus* species, with quantitative outcomes reported for growth, physiology, and/or remediation.

Inclusion criteria

Studies were included if they met all of the following criteria:

1. *Miscanthus*-specific results were included even if other crops were in the experiment.
2. Original experimental research focusing on applied treatments not genetics, breeding, or molecular characterisation.
3. Applied at least one PGR, BS, or GPM (single or combined).
4. Reported quantitative outcomes related to plant growth, biomass accumulation, physiological performance, and/or remediation efficiency
5. Published between 2010 and 2025 in a peer-reviewed journal.

Note, studies were excluded if they were reviews, opinion pieces, conference abstracts, or modelling-only studies.

Data extraction

For each included study, detailed information was extracted into an excel spreadsheet. Extracted variables included bibliographic details, experimental design characteristics, treatment descriptions, outcome domains, and reported effect directions.

Key extracted fields included:

1. *Miscanthus* species and genotype
2. Planting material (rhizome, plug, seed, stem)
3. Growth environment (growth chamber, greenhouse, pots, field, mixed)
4. Soil context (non-contaminated, marginal, contaminated)
5. Study duration and time-bin classification
6. Intervention class (biostimulant, microorganism, PGR, or combinations)
7. Application frequency, route, and developmental stage
8. Growth, physiological, and remediation outcomes
9. Direction of reported effects (increase, decrease, mixed, no effect)

The complete extracted dataset (29 studies and all coded variables) is provided as Supplementary Material (Table S1), enabling transparency and reuse.

Coding framework and outcome classification

To enable synthesis across heterogeneous study designs, outcomes were grouped into three high-level domains:

1. Growth outcomes, encompassing morphological traits and biomass accumulation (fresh or dry weight).
2. Physiological outcomes, including photosynthetic performance, antioxidant activity, nutrient status, and plant growth regulation indicators.

3. Remediation outcomes, including metal uptake, bioconcentration factors (BCF), translocation factors (TF), and soil contaminant dynamics.

Within each domain, the direction of effect was coded based on the authors' reported statistical comparisons relative to untreated controls. Effects were classified as increase, decrease, mixed (both positive and negative responses across parameters), no effect, or not assessed. This approach preserved author-reported interpretations while allowing cross-study comparison without imposing quantitative thresholds inappropriate for a scoping review.

Evidence mapping and data synthesis

Extracted data were analysed descriptively and visualised using evidence maps to illustrate research density, methodological clustering, and knowledge gaps. Heatmaps, stacked bar charts, and proportional plots were generated to summarise study distributions across species, planting material, environment, soil context, application strategy, and outcome domains. Figures derived from the evidence mapping are presented in Results, with corresponding methodological context referenced here.

RESULTS

Study Selection and Evidence Assembly

The study selection process is summarised in Figure 1, which outlines database searching, screening, and deduplication steps. Searches across Web of Science, Scopus, and PubMed initially identified 252 records. Following title screening, abstract screening and subsequent removal of duplicates, 29 studies met all inclusion criteria and were retained for synthesis. These studies form the evidence base for all subsequent analyses.

Distribution of Outcome Domains Across Intervention Classes

The distribution of studies across outcome domains and intervention classes is shown in Figure 2. Growth-related outcomes were the most frequently reported across all intervention classes, with particularly high representation in studies applying GPM, BS, and combined microbial–biostimulant treatments (GPM + BS). Physiological outcomes were less consistently assessed and were unevenly distributed across intervention

classes. Remediation-related outcomes were reported in a smaller subset of studies, primarily those conducted on contaminated or marginal soils and often in conjunction with growth measurements.

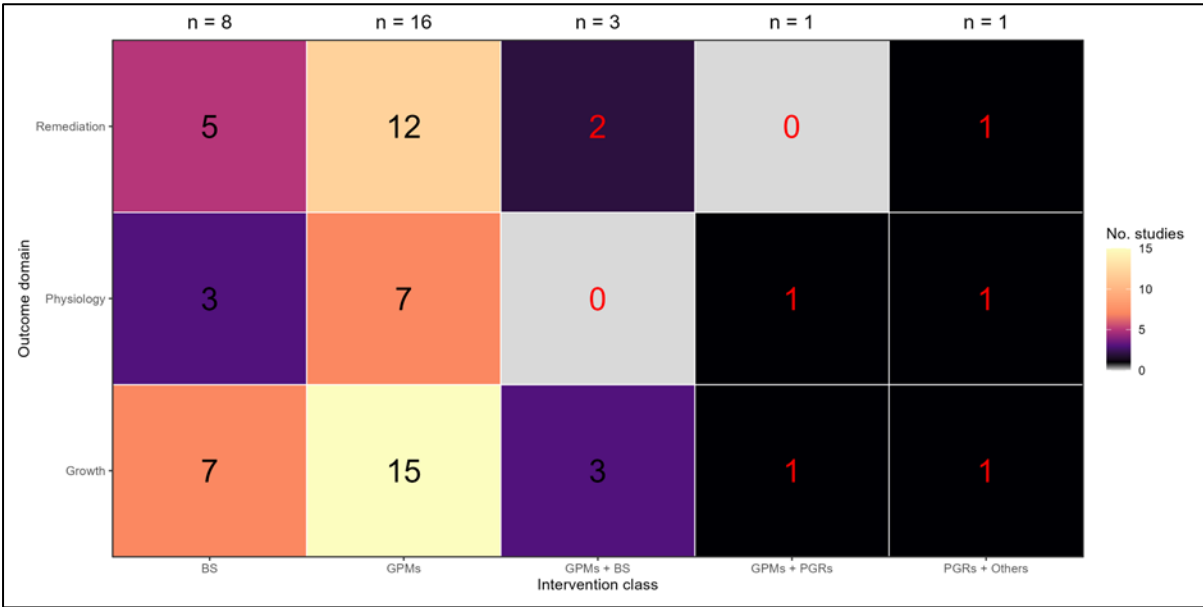


Figure 2. Distribution of outcome domains across intervention classes. Heat map showing the number of studies reporting growth, physiological, and remediation outcomes across five intervention classes: biostimulants (BS), growth-promoting microorganisms (GPM), combined GPM–biostimulant treatments (GPM + BS), GPM–plant growth regulator combinations (GPM + PGR), and plant growth regulator–other combinations (PGR + Others). “Others” refers to interventions combined with PGRs that were not classified as GPM or BS. Cell values indicate the number of studies reporting outcomes within each outcome domain and intervention class. Values shown at the top of each intervention-class column indicate the total number of studies within that class. Growth-related outcomes were most frequently reported across the intervention classes, whereas physiological and remediation outcomes were less consistently assessed.

Species, Planting Material, and Experimental Context

The distribution of species, planting material, experimental environment, soil context, study duration, application route, and application stage is presented in Figure 3. *Miscanthus x giganteus* was the most frequently studied taxon, followed by *Miscanthus sinensis* and *Miscanthus floridulus*, with relatively few studies on *Miscanthus sacchariflorus* and *Miscanthus lutarioriparius*. Rhizomes were the dominant planting material, while plugs and seed or stem-derived materials were less commonly used.

Most studies were conducted under controlled or semi-controlled conditions, including greenhouse, growth chamber, and pot-based outdoor experiments. Field studies were comparatively scarce and were typically associated with longer experimental durations. The evidence base was skewed towards studies conducted under contaminated or marginal soil conditions, with remediation-focused research substantially more

represented than studies evaluating *Miscanthus* growth and establishment under conventional agricultural conditions.

Patterns observed from the data base (Table S1) indicate that plug-and-seed-based studies are concentrated in controlled environments and shorter study durations, whereas rhizome-based studies span a wider range of environments, including pot trials and field experiments. This reflects prevailing experimental practices rather than tested causal relationships between planting material and environment.

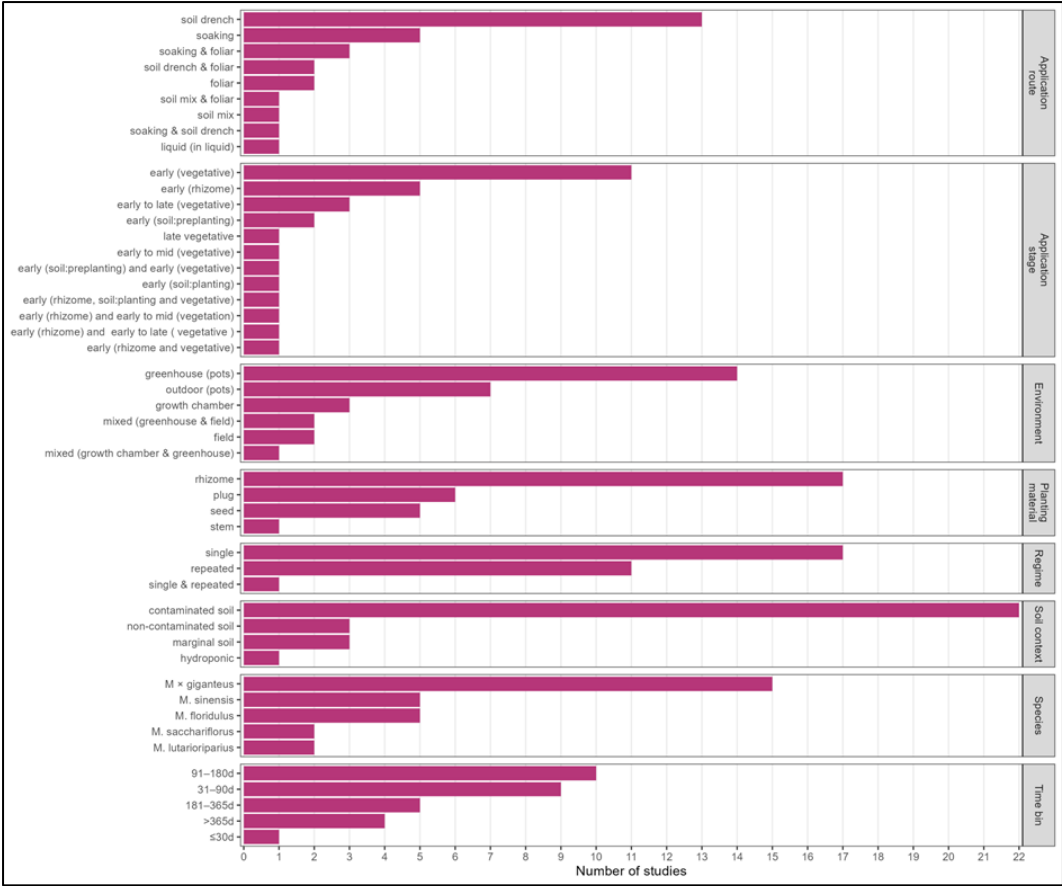


Figure 3. Characteristics of the evidence base across experimental dimensions. Multi-panel bar plots summarise the distribution of studies across eight experimental dimensions: application route, application stage, environment, planting material, regime, soil context, species, and time bin. The figure highlights the predominance of *Miscanthus x giganteus*, rhizome-based planting material, controlled or semi-controlled environments, contaminated or marginal soils, short- to medium-term study durations, and early-stage applications delivered primarily via soil drenching or soaking.

Intervention Application Strategies

Application strategies, including route, timing, and frequency, are summarised in Figure 3, which shows that soil drenching and soaking were the most common application routes, particularly for GPM and BS. Foliar applications were less frequent and were

primarily associated with PGR treatment. Applications were most commonly implemented at pre-planting or early vegetative stages, with relatively few studies applying treatments at later developmental stages.

Single applications were the most common approach, followed by repeated applications. Mixed application regimes were rare. These patterns underscore the strong emphasis on early establishment and initial growth responses within the current evidence base.

Direction of Effects Across Outcome Domains

The direction of reported effects across growth, physiological, and remediation domains is synthesised in Figure 4. For growth-related outcomes, most studies reported increases relative to untreated controls, particularly for biomass accumulation and morphological traits. Neutral or negative growth responses were less frequent and typically associated with specific soil constraints, late-stage applications, or interaction effects between treatments and environments.

Physiological outcomes exhibited greater heterogeneity. While several studies reported improvements in photosynthetic performance, antioxidant activity, or stress-related indicators, others reported no effect or mixed responses when multiple physiological parameters were assessed concurrently. Mixed classifications reflect simultaneous increase and decrease across different physiological indicators within the same study rather than contradictory findings. Additionally, more than 50 % of the evidence base did not report on physiological outcomes.

Remediation-related outcomes were most frequently classified as increased or mixed. Positive effects generally involved enhanced metal uptake, stabilisation, or improved phytoremediation indices, whereas mixed effects reflected trade-offs between metal accumulation and plant growth or physiological performance.

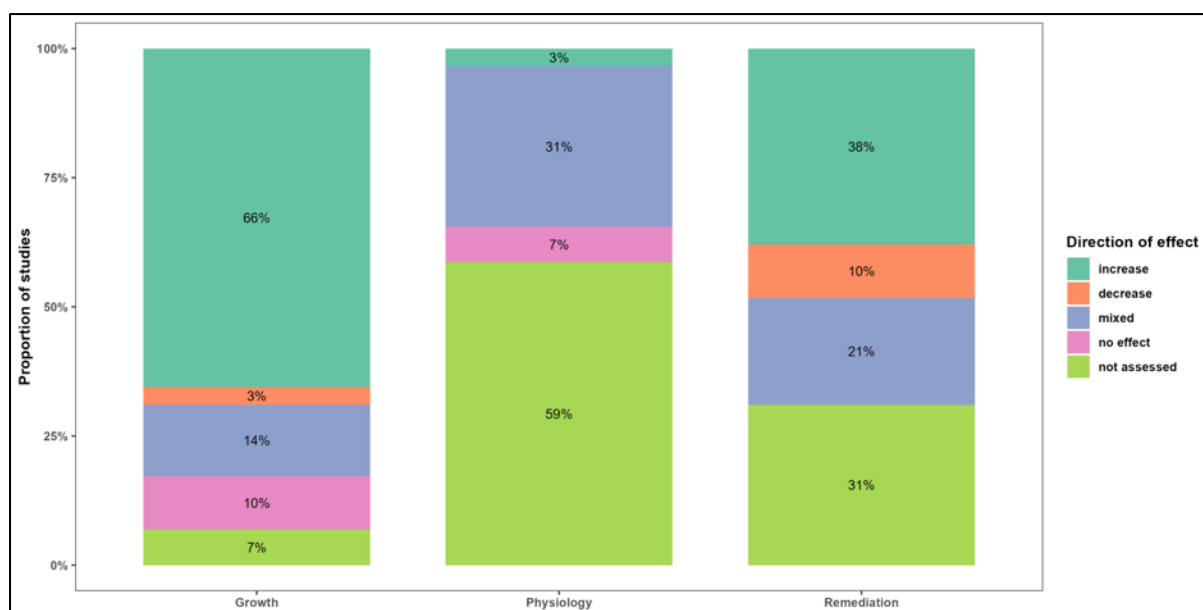


Figure 4. Direction of reported effects across outcome domains.

Stacked bar chart showing the proportional distribution of reported effect directions (increase, decrease, mixed, no effect, not assessed) across growth, physiological, and remediation outcome domains. Percentages are calculated relative to the total number of studies reporting each domain. Growth outcomes were most frequently associated with increases relative to controls, whereas physiological and remediation domains exhibited greater heterogeneity, including mixed and unassessed responses reflecting multi-parameter measurements and domain-specific trade-offs.

Temporal Scale of Experiments

Study durations were predominantly short- to medium-term, as shown in Figure 3, with most experiments conducted over ≤ 90 days or 91–180 days. Fewer studies extended beyond one year, and only a small number assessed multi-year responses under field conditions. Longer-duration studies more frequently addressed remediation performance and field relevance, whereas short-term experiments were typically focused on physiological or early growth responses.

Summary of Evidence Patterns

Taken together, the figures indicate that research on biologically mediated enhancement of *Miscanthus* performance is dominated by controlled-environment studies targeting early growth responses, with GPM and BS as the most frequently investigated interventions. Growth outcomes are well represented across the evidence base, whereas physiological and remediation outcomes are less consistently assessed and show a more variable reported direction of effect.

DISCUSSION

Structural biases in experimental design and their implications for *Miscanthus* optimisation

Across the reviewed literature, clear structural patterns emerge that shape *Miscanthus*' response to PGR, BS, and GPM. These patterns are not incidental but reflect entrenched experimental conventions that influence both the interpretation and transferability of reported outcomes. As shown in Figure 3, the literature is dominated by studies on *Miscanthus x giganteus*, with comparatively limited representation of seed-propagated species such as *Miscanthus sinensis* and *Miscanthus floridulus* or seeded hybrids of both. This imbalance reflects the historical prominence of *Miscanthus x giganteus* in European bioenergy research due to its high biomass potential and sterility, but it also constrains broader inference, particularly for seed-based or plug-derived establishment systems that are increasingly relevant to scalable deployment (Clifton-Brown *et al.*, 2019).

The choice of planting material further reinforces this bias. Rhizome-based systems dominate the evidence base (Figure 3), despite their well-documented limitations in cost, logistics, and scalability (Lewandowski *et al.*, 2016). In contrast, plug, stem, and seed-derived systems are underrepresented and are largely confined to controlled or semi-controlled environments, even though these pathways are potentially central to reducing establishment costs and enabling rapid area expansion. Research on plug establishment has demonstrated that early developmental stages are critical determinants of long-term stand performance, particularly under marginal or contaminated soil conditions (Wu *et al.*, 2022). The limited incorporation of such systems into PGR, BS and GPM trials, therefore, represents a significant disconnect between experimental research and applied deployment.

The experimental environment further narrows the scope of inference. The predominance of controlled-environment (CE) studies, including glasshouse and growth-chamber experiments (Figure 3), reflects the need to control environmental conditions when investigating hormonal and microbial interactions. However, the environmental conditions imposed in CE studies can differ substantially from those

experienced under field conditions. In particular, temperature and soil moisture may fluctuate differently in pots and controlled environments than in field soils, while microbial communities and competitive interactions are also likely to differ (Pidlisnyuk *et al.*, 2020; Peroni *et al.*, 2025). These differences may influence plant–soil–microbe interactions and treatment responses, thereby limiting the direct transfer of findings from controlled environments to field conditions. Consequently, treatment effects observed under CE conditions cannot necessarily be assumed to translate directly to field conditions, particularly where responses depend on temperature, soil moisture, microbial communities, or other environmental factors encountered during establishment.

Time scale constitutes an additional constraint. As illustrated in Figure 3, most studies are short-term, frequently concluding within one growing season or less. While this approach captures acute physiological and growth responses, it is poorly aligned with the perennial nature of *Miscanthus*. Although establishment success, overwinter survival, and multi-year biomass accumulation are central to bioenergy viability and carbon mitigation potential (Hastings *et al.*, 2017), but these factors remain insufficiently addressed in PGR-focused research. Collectively, these structural biases frame the existing evidence base and must be considered when interpreting reported treatment effects.

Growth-dominated outcomes and the limits of biomass-centric optimisation

Growth and biomass accumulation represent the most frequently reported response variables across the reviewed studies, reflecting the central role of yield in bioenergy crop evaluation. This emphasis is evident in the dominance of growth outcomes across intervention classes (Figure 2) and is unsurprising given that biomass productivity is central to the economic viability and resource-use efficiency of *Miscanthus* deployment. However, biomass yield alone does not capture the full sustainability performance of the crop. Focusing predominantly on short-term growth responses may overlook other factors relevant to sustainable deployment, including resource requirements, soil impacts, environmental resilience, establishment success, and the persistence of treatment effects.

Many studies reported increases in shoot or total biomass following application of GPM or BS, particularly under contaminated or nutrient-poor soils (Babu *et al.*, 2015; Fei *et al.*, 2020; Nurzhanova *et al.*, 2023). These responses are often attributed to enhanced nutrient acquisition, hormonal stimulation, or improved stress tolerance. Nevertheless, some studies also documented neutral or negative growth responses despite measurable physiological changes (Malinska *et al.*, 2020; Schmidt *et al.*, 2017). Such outcomes indicate that physiological modulation does not necessarily translate into biomass gains within short experimental windows.

Importantly, growth responses must be interpreted in the context of the establishment stage and management objective. Early-stage biomass increases may reflect transient stimulation rather than durable yield enhancement. Work on *Miscanthus* establishment has demonstrated that early vigour does not always correlate with long-term stand productivity, particularly where belowground allocation or overwintering capacity is compromised (Ashman *et al.*, 2023b; Clifton-Brown *et al.*, 2019). The dominance of early growth metrics therefore limits conclusions about longer-term crop performance and its potential contribution to climate mitigation. Biomass yield, soil carbon dynamics and cultivation practices all influence the overall greenhouse-gas balance of *Miscanthus*, but robust assessment of these outcomes requires measurements over longer time periods than are typically captured in early-stage experiments.

Physiological modulation and remediation trade-offs

Physiological and remediation responses introduce additional complexity into the interpretation of treatment effects. As shown in Figure 4, studies frequently reported mixed or context-dependent physiological outcomes, particularly for photosynthetic performance and oxidative stress markers. These responses often reflect trade-offs between growth stimulation, stress mitigation, and contaminant uptake.

Some studies demonstrated enhanced antioxidant activity and chlorophyll content following GPM or PGR application, particularly under heavy-metal stress (Guo *et al.*, 2017; Wang *et al.*, 2023). Such responses are consistent with improved stress-tolerance mechanisms, including reactive oxygen species scavenging and hormonal regulation. However, enhanced physiological performance did not uniformly coincide with

increased biomass, underscoring the decoupling between stress mitigation and growth under certain conditions.

Remediation outcomes further complicate interpretation. Increases in metal uptake or translocation were often accompanied by growth penalties or neutral biomass responses (Lutts *et al.*, 2025; Pidlisnyuk *et al.*, 2022). Conversely, treatments that stabilised contaminants within root tissues or reduced bioavailability sometimes supported biomass production but limited phytoextraction efficiency (Babu *et al.*, 2015; Sarkar *et al.*, 2018). These patterns indicate that “mixed” outcomes do not reflect experimental inconsistency but rather divergent optimisation pathways, namely phytostabilisation versus phytoextraction.

This distinction is critical for net-zero deployment. Systems prioritising clean biomass production may favour stabilisation strategies that minimise contaminant transfer to harvestable tissues, whereas remediation-focused systems may accept reduced biomass in exchange for accelerated contaminant removal. The reviewed literature frequently conflates these objectives, highlighting the need for clearer framing of intended outcomes in future studies.

Relevance to the net-zero bioenergy systems

Taken together, the reviewed evidence indicates that while PGR, BS, and GPM can enhance *Miscanthus* performance under marginal and contaminated conditions, their effectiveness is strongly contingent on experimental context, management objective, and temporal scale. Short-term growth stimulation, though frequently reported, does not necessarily equate to sustainable yield enhancement or long-term carbon mitigation.

For *Miscanthus* to contribute meaningfully to net-zero targets, optimisation strategies must align with scalable establishment systems, long-term productivity, and biomass-quality constraints. Overreliance on rhizome-based, short-duration, controlled-environment studies limits the applicability of current findings to real-world deployment. Greater emphasis on plug- and seed-derived systems, multi-year field trials, and integrated assessment of growth, physiology, and remediation is required to bridge this gap.

Moreover, the frequent occurrence of mixed outcomes underscores the need to move beyond binary classifications of treatment success. Instead, optimisation should be evaluated relative to defined system goals, whether biomass production, soil rehabilitation, or combined phytomanagement. Without such clarity, reported treatment effects risk being misinterpreted or overgeneralised.

CONCLUSION

This scoping review synthesised fifteen years of experimental research on the use of PGR, BS, and GPM to optimise *Miscanthus* performance under marginal and contaminated soil conditions. The evidence indicates that such interventions can enhance early growth, modulate physiological stress responses, and influence remediation outcomes; however, their effectiveness is highly context-dependent, while structural biases in experimental design constrain the interpretation and generalisability of these effects.

Across the literature, a strong reliance on *Miscanthus* × *giganteus*, rhizome-based establishment, controlled environments, and short experimental durations limits the generalisability of reported outcomes to field-scale, long-term bioenergy systems. While growth and biomass responses dominate reported outcomes, physiological and remediation responses frequently exhibit mixed or trade-off-driven patterns, reflecting divergent optimisation objectives rather than experimental inconsistency. These findings highlight the need to interpret intervention responses across multiple dimensions, including establishment strategy, persistence of effects over time, and the intended function of the intervention, rather than relying predominantly on short-term biomass responses.

For *Miscanthus* to contribute meaningfully to net-zero targets, future research must better align experimental design with deployment realities. This includes greater emphasis on plug- and seed-derived establishment systems, multi-year field trials, and explicit differentiation between biomass-oriented and remediation-oriented management goals. By addressing these gaps, optimisation strategies based on PGR, BS, and GPM can be more effectively translated from experimental settings into scalable, resilient bioenergy systems capable of delivering sustained yield and environmental co-benefits.

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Supplementary materials

Documents by year

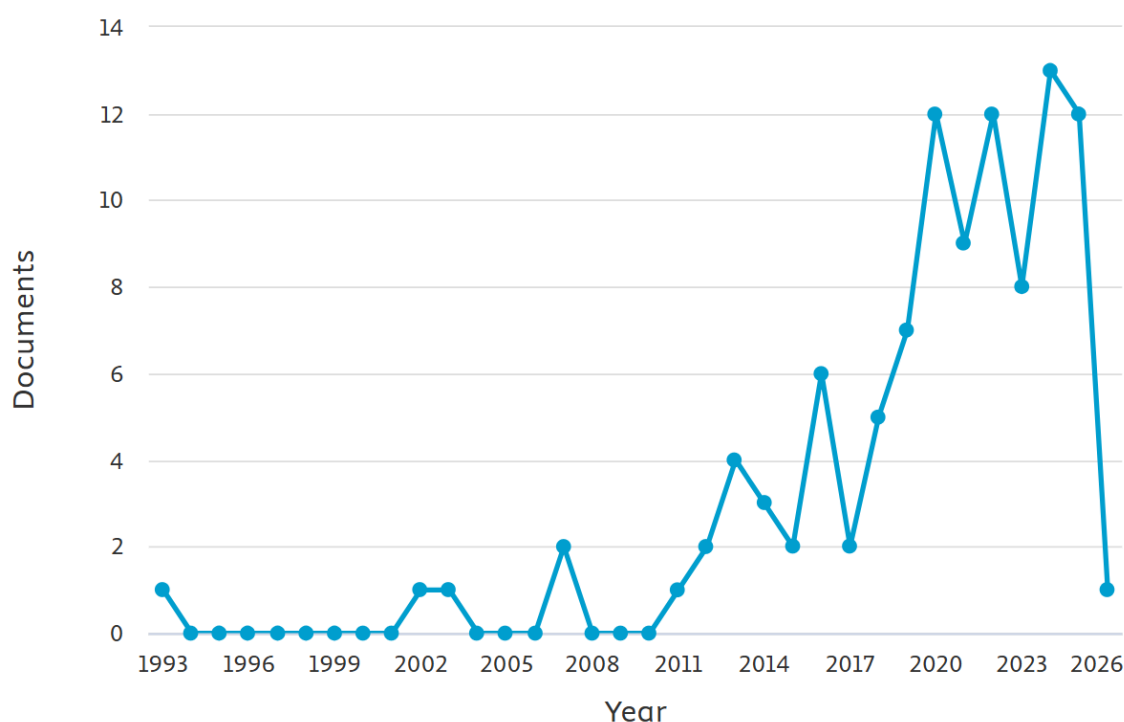


Figure S1. Yearly publication output retrieved using the Scopus search string for *Miscanthus* and plant growth regulators (1993–2025). Line graph showing the number of documents published per year based on the Scopus search combining *Miscanthus* and plant growth regulators. Publication output remained low from 1993 to 2010, followed by a steady increase after 2011 and a sharp rise from 2017 onward, peaking between 2020 and 2025. The x-axis extends to 2026 for visual continuity, although publications from 2026 were not included in the review.

DATABASE SEARCH STRING

Web of Science (WoS)

TS=("Miscanthus" OR "Miscanthus giganteus" OR "Miscanthus sinensis")
AND TS=("plant growth regulator*" OR "growth promot*" OR "growth retard*" OR
"biostimulant*" OR "auxin*" OR "indole-3-acetic acid" OR "IAA" OR "cytokinin*" OR "zeatin" OR "kinetin"
OR "gibberellin*" OR "GA3" OR "abscisic acid" OR "ABA" OR "ethylene" OR "ethephon"
OR "brassinosteroid*" OR "brassinolide" OR "salicylic acid" OR "jasmonic acid" OR
"methyl jasmonate")
AND TS=(growth OR yield OR biomass OR "plant height" OR tiller* OR "leaf area"
OR photosynth* OR chlorophyll OR productivity OR development)
AND LANGUAGE: (English)
Timespan=2010-2025
Document Types=(Article)

PubMed

("Miscanthus"[Title/Abstract] OR "Miscanthus giganteus"[Title/Abstract] OR
"Miscanthus sinensis"[Title/Abstract])
AND ("plant growth regulator"[Title/Abstract] OR "plant growth
regulators"[Title/Abstract])

OR "growth promot*" [Title/Abstract] OR "growth retard*" [Title/Abstract] OR
 biostimulant* [Title/Abstract]
 OR auxin* [Title/Abstract] OR "indole-3-acetic acid" [Title/Abstract] OR
 IAA [Title/Abstract]
 OR cytokinin* [Title/Abstract] OR zeatin [Title/Abstract] OR kinetin [Title/Abstract]
 OR gibberellin* [Title/Abstract] OR GA3 [Title/Abstract] OR "abscisic acid" [Title/Abstract]
 OR ABA [Title/Abstract] OR ethylene [Title/Abstract] OR ethephon [Title/Abstract]
 OR brassinosteroid* [Title/Abstract] OR brassinolide [Title/Abstract]
 OR "salicylic acid" [Title/Abstract] OR "jasmonic acid" [Title/Abstract] OR "methyl
 jasmonate" [Title/Abstract])
 AND (growth [Title/Abstract] OR yield [Title/Abstract] OR biomass [Title/Abstract]
 OR "plant height" [Title/Abstract] OR tiller* [Title/Abstract] OR "leaf area" [Title/Abstract]
 OR photosynth* [Title/Abstract] OR chlorophyll [Title/Abstract] OR
 productivity [Title/Abstract]
 OR development [Title/Abstract])
 AND ("2010" [Date - Publication] : "2025" [Date - Publication])

Scopus

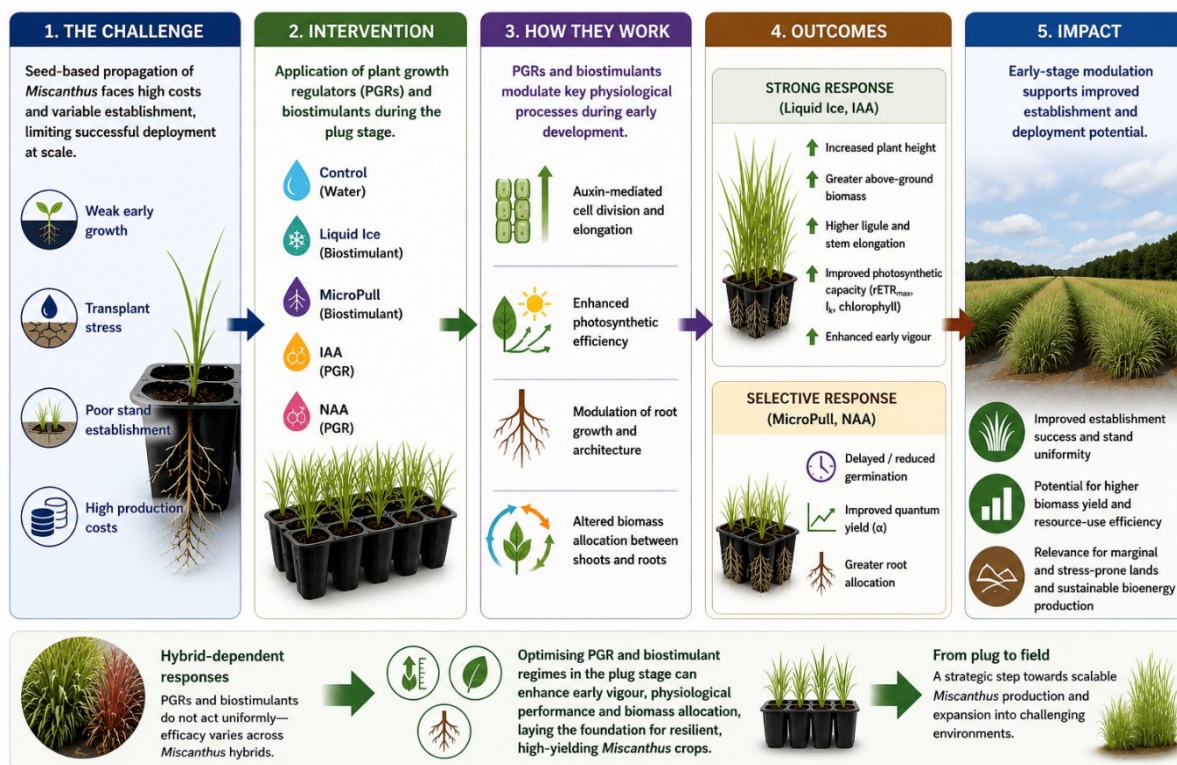
TITLE-ABS-KEY("Miscanthus" OR "Miscanthus giganteus" OR "Miscanthus sinensis")
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 biostimulant*
 OR auxin* OR "indole-3-acetic acid" OR IAA OR cytokinin* OR zeatin OR kinetin
 OR gibberellin* OR GA3 OR "abscisic acid" OR ABA OR ethylene OR ethephon
 OR brassinosteroid* OR brassinolide OR "salicylic acid" OR "jasmonic acid" OR "methyl
 jasmonate")
 AND TITLE-ABS-KEY(growth OR yield OR biomass OR "plant height" OR tiller* OR "leaf
 area"
 OR photosynth* OR chlorophyll OR productivity OR development)
 AND (LIMIT-TO (PUBYEAR, 2025) OR LIMIT-TO (PUBYEAR, 2024) OR LIMIT-TO (PUBYEAR,
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 AND (LIMIT-TO (DOCTYPE, "ar"))
 AND (LIMIT-TO (LANGUAGE, "English"))

Table S1. Data Extraction Table (The datasets supporting this review are archived in an Open Science Framework (OSF) repository. The repository will be made publicly available upon publication of the associated manuscript. – [CLICK HERE](#))

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	A				
sn_1	Authors_1	Publicatic Source	Title DOI	database	species	material	Environm	soil	conte	time	bin	class	type	applied	route	stage	growth	ou	growth	do	growth	eff	physiol	physiol	physiol	remediat	remediat	remediat	field	relevance
1	Anam, A. C.	2015	Journal of 10.1016/j.jscopus	M.sin	rhizome	outdoor (j	contamin:91-180d	M	Pseudom	single	soil drenc	early (soil	Shoot and Biomass i	increase	chlorophy	photosynt	mixed	As, Cd, Cu	Y	increase	N									
2	Fel, HM; C	2020	CANADIAN 10.1139/cj WoS	Mxg	rhizome	mixed (gre	marginal: 31-90d	M-B	PGPR con	single & re	soil drenc	early (rhiz	shoot bio	Biomass i	increase	none	none	not acces	none	N	not acces	Y								
3	Guo, HP; C	2017	ECOTOXIC 10.1016/j.j WoS	M.sac	seed	growth ch	hydroponi:31-90d	B	Malic acic	single	liquid (in l	early (vegi	shoot and	Biomass i	increase	net photo	photosynt	mixed	Cd	Y	decrease	N								
4	Haruma, T	2021	LETTERS IF 10.1111/la WoS	M.sin	seed	growth ch	contamin:31-90d	M	Phialoce	single	soil drenc	early (vegi	root lengt	Biomass i	increase	Chloroger	nutrient &	mixed	Cu, Fe, P	Y	mixed	N								
5	Iqbal, Y; M	2025	INDUSTRIU 10.1016/j.j WoS	M.lut	rhizome	field	contamin: >365d	B	humic aci	repeated	soil drenc	early (vegi	plant heig	Biomass i	increase	none	none	not acces	Pb, Cd, Cu	Y	mixed	Y								
6	Janczak- P	2024	AGRICULTU 10.3390/a WoS	M.sin	seed	greenhou	contamin:181-365d	B	Bombard	single	foliar	late vege	none	none	not acces	SPAD, Fv	photosynt	increase	none	N	not acces	N								
7	Liu, SM; Li	2021	PLANTS-B 10.3390/pl WoS	M.flo	plug	outdoor (j	contamin:31-90d	M	Lelliotia	j	single	soil drenc	early (vegi	plant heig	Biomass i	increase	none	none	not acces	Cd, Bioc	Y	increase	N							
8	Liu, Y; Lud	2020	INDUSTRIU 10.1016/j.j WoS	Mxg	stem	mixed (gre	non-cont:91-180d	M	Herbaspl	single	soaking	early (rhiz	plant heig	Biomass i	increase	none	none	not acces	none	N	not acces	Y								
9	Lu, XY; Yai	2024	JOURNAL I 10.1080/1 WoS	M.sin	seed	growth ch	contamin:31-90d	M	Talaromy	single	soil drenc	early (vegi	height, Nc	Biomass i	mixed	none	none	not acces	Cd, Cu, MY		mixed	N								
10	Lutts S, Re	2025	Chemospl 10.1016/j.j PubMed	Mxg	rhizome	mixed (grc	contamin:91-180d	P+O	trans-zeat	repeated	foliar	early to m	height, no	Biomass i	mixed	Pn, Ci, E, g	photosynt	mixed	Cd, Pb, Zi	Y	increase	N								
11	Malinska,	2020	PLANTS-B 10.3390/pl WoS	Mxg	rhizome	outdoor (j	marginal: 181-365d	B	Stimpo an	repeated	soaking &	early (rhiz	no. stoma	Morpholo	decrease	OLKIIP, Fv	photosynt	no effect	none	N	not acces	N								
12	Nebeska,	2019	REVIEWS C 10.1515/re WoS	Mxg	rhizome	greenhou	contamin:181-365d	B	Stimpo an	repeated	soaking &	early (rhiz	no.stem, i	Biomass i	increase	none	none	not acces	Cr, Mn, N	Y	mixed	N								
13	Nurzhano	2023	ENVIRONF 10.1007/s WoS	Mxg	rhizome	greenhou	contamin:181-365d	M	Trichospo	single	soil drenc	early (soil	plant heig	Biomass i	increase	chlorophy	photosynt	mixed	As, Pb, Zi	Y	increase	N								
14	Ofori-Agyi	2024	Science of 10.1016/j.j Scopus	Mxg	plug	greenhou	contamin:31-90d	M-B	Symbi	vi (i	repeated	soil drenc	early to la	shoot bio	Biomass i	increase	none	none	not acces	Cd, Pb, ai	Y	increase	N							
15	Peroni, P.;	2024	Plants 10.3390/pl Scopus	M.lut	rhizome	greenhou	contamin:91-180d	M-B	Symbi	vi (i	repeated	soil drenc	early to la	shoot heig	Biomass i	increase	none	none	not acces	Cd and Cu	Y	increase	N							
16	Peroni, P.;	2025	INDUSTRIU 10.1016/j.j WoS	Mxg	rhizome	field	contamin: >365d	B	Lonite (h	c	repeated	soil drenc	early to la	shoot heig	Biomass i	mixed	none	none	not acces	BCF, SMU	Y	mixed	Y							
17	Pidlisnyuk	2022	AGRONOM 10.3390/a WoS	Mxg	rhizome	outdoor (j	contamin:91-180d	B	Kamethur	single	soaking	early (rhiz	plant heig	Biomass i	mixed	none	none	not acces	BCF, CBI	Y	decrease	N								
18	Pidlisnyuk	2022	INTERNAT 10.1016/j.j WoS	Mxg	rhizome	greenhou	contamin:181-365d	M	Bacillus a	single	soaking	early (rhiz	none	none	not acces	none	none	not acces	V, Cr, Mn	Y	decrease	N								
19	Pidlisnyuk	2022	AGRONOM 10.3390/a WoS	Mxg	rhizome	outdoor (j	contamin:91-180d	M	Stenotro	pl	single	soaking	early (rhiz	plant heig	Biomass i	no effect	none	none	not acces	TF, BCF, C	Y	mixed	N							
20	Pidlisnyuk	2022	APPLIED S 10.3390/a WoS	Mxg	rhizome	greenhou	marginal: >365d	B	Charkor, F	repeated	soaking &	early (rhiz	stem leng	Biomass i	increase	none	none	not acces	none	N	not acces	N								
21	Pranaw, K	2020	BIOLOGY- 10.3390/bi WoS	Mxg	rhizome	greenhou	contamin:91-180d	M	Bacillus a	single	soaking	early (rhiz	plant heig	Biomass i	increase	none	none	not acces	none	N	not acces	N								
22	Sarkar, A.;	2018	CHEMISTR 10.1080/0 WoS	M.sac	rhizome	greenhou	contamin:91-180d	M	Serakinko	single	soil mix	early (soil	biomass (	Biomass i	increase	Fv/Fm, chl	photosynt	mixed	Pb	Y	increase	N								
23	Schmidt, C	2018	WORLD JC 10.1007/s WoS	Mxg	rhizome	greenhou	contamin: >365d	M	long list o	single	soaking &	early (vegi	biomass (	Biomass i	no effect	none	none	not acces	none	N	not acces	Y								
24	Schmidt, C	2017	PLANT PH 10.1016/j.j WoS	Mxg	rhizome	greenhou	non-cont:91-180d	M-P	Rhizoph	a	single	soil mix &	early (soil	height, no	Biomass i	no effect	PA, E, Fv	photosynt	mixed	none	N	not acces	N							
25	Straub, Dr.	2013	JOURNAL I 10.1093/j WoS	M.sin	seed	greenhou	non-cont: <30d	M	Herbaspl	repeated	soil drenc	early (vegi	no. & leng	Biomass i	increase	TN, total p	nutrient &	mixed	none	N	not acces	N								
26	Wang, XT;	2023	SCIENCE C 10.1016/j.j WoS	M.flo	plug	greenhou	contamin:91-180d	M	Bacillus c	single	soil drenc	early (vegi	shoot heig	Biomass i	increase	photosynt	photosynt	mixed	Cd	Y	increase	N								
27	Wang, Y; J	2025	ECOTOXIC 10.1016/j.j WoS	M.flo	plug	outdoor (j	contamin:31-90d	M	Beauveria	single	soil drenc	early (vegi	plant heig	Biomass i	increase	none	none	not acces	Cd, TF, B	Y	increase	N								
28	Xiao, Y; M	2024	Environm 10.1007/s Scopus	M.flo	plug	outdoor (j	contamin:31-90d	M	Klebsiella	repeated	soil drenc	early (vegi	plant heig	Biomass i	increase	SOD, POD	photosynt	no effect	As, Cd, Cu	Y	increase	N								
29	Xiao, YH; I	2022	INTERNAT 10.1080/1 WoS	M.flo	plug	greenhou	contamin:31-90d	M	Klebsiella	repeated	soil drenc	early (vegi	plant heig	Biomass i	increase	none	none	not acces	Pb, BCF, Y		increase	N								

Chapter 3

Can Biostimulants and Phytohormones Improve Early Growth, Biomass Allocation, and Photosynthesis in *Miscanthus* Plug Plants?



PGR, plant growth regulator; IAA, indole-3-acetic acid; NAA, 1-naphthaleneacetic acid; $rETR_{max}$, maximum electron transport rate; I_0 , light saturation point; α , quantum yield.

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ABSTRACT

Seed-based propagation of *Miscanthus* offers scalable potential for expanding bioenergy production; however, early establishment remains a major limitation due to poor plug vigour and variable field performance. This study evaluated the influence of selected biostimulants and phytohormones on germination, growth, biomass allocation, and photosynthetic efficiency in three *Miscanthus* seed-based hybrids (GNT3, GNT14, and GNT43) grown under controlled glasshouse conditions. Five treatments were applied, including Liquid Ice, MicroPull, indole-3-acetic acid (IAA), 1-naphthaleneacetic acid (NAA), and an untreated control during the plug phase. ANOVA revealed strong hybrid-dependent responses. Liquid Ice and IAA significantly enhanced stem elongation, total biomass, and photosynthetic capacity ($rETR_{max}$, I_k), while also shifting biomass allocation toward above-ground structures. MicroPull, in contrast, delayed germination and reduced photosynthetic parameters in certain hybrids, though it improved initial quantum yield (α) at low light intensities. Enhanced root and bud development under Liquid Ice and NAA treatments suggests improved plug vigour and potential for stronger field establishment. The observed morphological and physiological modifications indicate that targeted PGR use can simultaneously enhance early growth, resource allocation, and photosynthetic performance in *Miscanthus*. Additionally, the findings highlight the importance of hybrid-specific optimization of PGR regimes to improve plug quality and establishment success, advancing the scalability of seed-based *Miscanthus* systems for sustainable bioenergy production and net-zero carbon targets.

KEYWORDS

Miscanthus plugs, Plant Growth Regulators, Biostimulants, Climate Change, Net-Zero, Clean Energy, Biomass, PCA.

INTRODUCTION

Miscanthus is a leading candidate in the pursuit of sustainable bioenergy, owing to its high biomass yield, low input requirements, and adaptability to diverse environmental conditions (Winkler *et al.*, 2020). As a C₄ perennial grass, *Miscanthus* offers considerable promise in meeting global renewable energy targets and advancing the transition to net-zero emissions (Clifton-Brown *et al.*, 2019). C₄ plants like *Miscanthus* are particularly efficient at sequestering atmospheric carbon, storing it in their below-ground biomass or contributing it to the soil through leaf litter and root turnover (Clifton-Brown *et al.*, 2007; Dang *et al.*, 2025). Since only the above-ground biomass is harvested annually, the sequestered carbon remains largely undisturbed in the soil (Dewi *et al.*, 2022). Furthermore, *Miscanthus* offers a highly cost-effective alternative to fossil fuels, up to 30 times cheaper in some cases (Hastings *et al.*, 2008; Wu *et al.*, 2021), and its utility extends beyond bioenergy, with applications across various industries, which is well documented in Shavyrkina *et al.* (2023).

Traditionally propagated via rhizomes, *Miscanthus* is now increasingly being developed through seed-based hybrids, which offer several advantages such as scalability, genetic diversity, and potential in reducing establishment cost compared to rhizome-based propagation (Lewandowski *et al.*, 2016; Clifton-Brown *et al.*, 2017), although the increased diversity may result in greater variability in early growth and establishment. Also, direct sowing of seeds into the field often results in poor establishment, particularly in temperate regions where environmental stress can hinder early seedling development (Clifton-Brown *et al.*, 2017; Ashman *et al.*, 2018). To overcome this, raising seedlings as plugs in controlled environments prior to field transplanting has become a widely accepted alternative. This approach has been shown to achieve up to 95% establishment success, compared to approximately 20% from direct sowing (Hastings *et al.*, 2017; Ashman *et al.*, 2018). However, plug-based systems remain constrained by the resource demands of greenhouse production, including energy, labour, substrate, and transport requirements, which collectively contribute to increased establishment costs (Wu *et al.*, 2021). This reflects a fundamental trade-off in plug propagation: extended nursery cultivation improves seedling size and establishment reliability but increases input costs.

Optimising this balance remains a key challenge in scaling *Miscanthus* plug production (Clifton-Brown *et al.*, 2017; Ashman *et al.*, 2023b).

Plug production has suffered some setbacks in deployment, particularly relating to variability in germination and early seedling establishment. These issues may arise from factors such as seed maturity at harvest, threshing damage, storage conditions and duration, and sowing accuracy (Clifton-Brown *et al.*, 2017). Beyond this, plug-grown plants, although more physiologically advanced than dormant rhizomes, can be more vulnerable to climatic extremes due to their active metabolism. These plugs require rapid root extension into the surrounding soil to access moisture, which is critical for early establishment success (Ashman *et al.*, 2023a). To safeguard against soil moisture loss and improve plug-to-field contact, mulch film has become essential for first-year survival, but it also adds considerable material and labour costs (Ashman *et al.*, 2023a). Additionally, to withstand increasingly erratic winters and ensure second-year emergence, early development of a robust rhizome during the plug stage or in the first year is vital; a significant agronomic challenge under climate stress (Zheng *et al.*, 2021).

Current research efforts are focused on optimising the performance of these plugs. For instance, Wu *et al.* (2021) explored techniques such as supplemental lighting, heating, and variation in compost type and volume to improve plug development. Despite these advances, there remains room for improvement, especially in enhancing plug vigour and transplant success. One promising strategy lies in the application of biostimulants and phytohormones, which are known to influence key physiological processes such as root development, stress tolerance, and growth rate (Jańczak-Pieniżek *et al.*, 2023). While auxin, gibberellin, cytokinin, and other compounds have shown potential in boosting plant establishment, most studies in *Miscanthus* have focused on using them in rhizome propagation. For example, Fei *et al.* (2020) demonstrated improved biomass production through the use of soil microbes and biostimulants, and Katelevskij *et al.* (2020) reported increases in height, shoot number, biomass, and energy output. In contrast, Pidlisnyuk *et al.* (2022) observed mixed results, with only one out of three plant growth regulator treatments producing a positive response.

Though limited in *Miscanthus*, research from other crops provides supporting evidence for the use of growth regulators at the seedling stage. Tang *et al.* (2024) found that pre-

transplant treatment of rice seedlings (plugs) with mixed growth regulators enhanced root systems and yield, particularly when applied ten days before transplanting. Similarly, Li *et al.* (2016) reported reduced transplant shock in rice seedlings pre-treated with triacontanol, owing to improved growth and antioxidant responses. Other studies have explored the use of growth retardants to control seedling overgrowth; for example, Duong *et al.* (2024) showed that prohexadione-calcium significantly reduced height and leaf area in maize, a C₄ annual grass, making it a potential tool for managing seedling size pre-transplant.

In this context, the application of biostimulants and phytohormones during the plug stage presents a promising strategy to enhance seedling vigour, promote early growth, and improve the post-transplant establishment of *Miscanthus*. While their benefits have been demonstrated in other crops, their effects on *Miscanthus* plugs remain underexplored. This study aims to evaluate the potential of such treatments to improve plug performance by assessing key growth parameters, including germination, biomass accumulation, root and shoot development, and photosynthetic efficiency. Through this investigation, the research seeks to contribute to the optimisation of plug-based propagation systems for *Miscanthus*, ultimately supporting its scalability and role in sustainable bioenergy production and climate mitigation efforts.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The plant materials used in this study were three seed-based *Miscanthus* × *giganteus* J.M. Greif & Deuter ex Hodk. & Renvoize hybrids (*M. sacchariflorus* × *M. sinensis*). These hybrids were GNT3 (Artemis), GNT14 (Brontes), and GNT43 (Aphrodite) and were all derived from the Giant-link *Miscanthus* project. Full information for each hybrid is available in the [CPVO Register](#) under their respective denomination names (Artemis, Brontes, and Aphrodite). These hybrids are commercially relevant hybrids and were provided by Terravesta Ltd (a UK-based company specialising in the commercial development and supply of *Miscanthus* for sustainable biomass production). This research was carried out in the research glasshouse of Liverpool John Moores University (53°24'45.1"N, 2°58'47.7"W). Throughout the experiment, the glasshouse temperature and humidity averaged 19°C and 50%, respectively. A 16/8-hour light/dark cycle was

achieved using supplementary Kroptek KP4 broad-spectrum LED lighting, to compensate for the limited natural daylight conditions in winter months. The seeds were sown on compost (Levington Multipurpose Compost) in 12-celled seed trays (Mixceco). Three seeds were sown per cell, and upon successful germination, they were thinned to one seedling per cell. They were sown on January 26, 2024, and the lids (supplied with the trays) were left on for two weeks after sowing to maintain a humid environment, which is essential for *Miscanthus* seed germination.

Experimental Design

A split-plot randomised complete block design (RCBD) was used to minimize environmental variation (randomisation was done in MS Excel using the RAND function). The experiment consisted of 6 blocks for each treatment, and each contained 4 replicates of the three hybrids (Figure 1). To further reduce positional effects within the growth environment, the six blocks were repositioned weekly, with the internal arrangement of trays within each block maintained, to provide more uniform exposure to light and temperature across the experimental area.

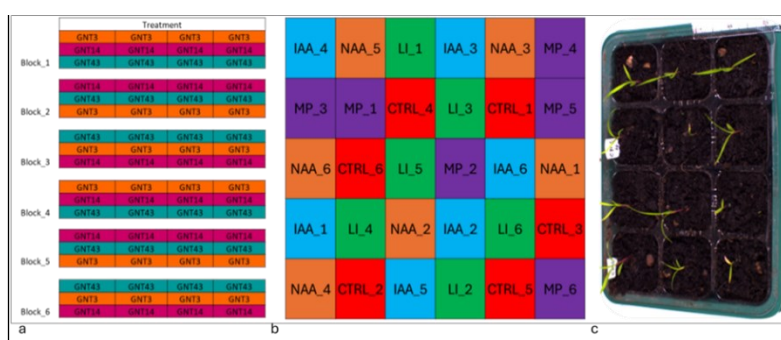


Figure 1. Panel (a) shows the layout of hybrids within each block of treatment; panel (b) maps the specific PGR treatment and replicate number to individual tray starting positions; panel (c) is a representative photograph of one complete block of the 12-celled trays.

Plant Growth Regulator Treatments

Seed trays received one of five watering treatments: untreated water (Control), Liquid Ice (LI), MicroPull (MP), indole-3-acetic acid (IAA) or 1-naphthaleneacetic acid (NAA). Selection of these plant growth regulators was informed by a one-week preliminary seed germination test and six weeks growth monitoring that identified these compounds as having promising effects on early-stage *Miscanthus* growth and development. Full list of Biostimulant and phytohormones tested is included as supplementary material (Table S2). Although the exact composition of Liquid Ice and MicroPull were not explicitly

disclosed by the suppliers, both were reported to contain microbial inoculants, humic acid, amino acid, hormone precursors, and trace micronutrients. These classes of compounds are commonly used in biostimulant formulations to enhance nutrient uptake, stress tolerance, and growth responses (Cristofano *et al.*, 2021; Kaushal *et al.*, 2023). Treatments were delivered by adding the solution to drain trays beneath each seed cell block. The two biostimulants Liquid Ice and MicroPull were applied at 0.5 mL L⁻¹ and 1 mL L⁻¹, respectively. The two auxin phytohormones IAA and NAA were applied at 2 mL L⁻¹, and 0.2 mg L⁻¹, respectively. All concentrations followed the manufacturers' recommended dose or half of it based on preliminary tests. Each treatment was administered at least once weekly for ten weeks, after which all trays received only water for the remainder of the study (6 more weeks).

Data Collection and Measurements

Growth monitoring: Stretched height (distance from stem base to the tip of the tallest leaf [Nunn *et al.*, 2017]) was measured weekly with a standard metric ruler.

Photosynthesis: Photosynthetic parameters were assessed using a FluorCam 800MF imaging fluorometer (Photon Systems Instruments, Drásov, Czech Republic) two weeks after germination. Measurements followed an adjusted version of the Rapid Light Curve (RLC) with far-red pre-illumination protocol described by Herdean (2022), optimized for *Miscanthus* plug experiments. Plants were kept in dark conditions for 30 minutes before the quenching kinetic analysis, to ensure maximum photochemical efficiency of photosystem II (PSII) (Murchie and Lawson, 2013).

For the quenching kinetic analysis, a 5 s baseline fluorescence (F_o) was recorded, followed by an 800 ms saturating pulse of approximately 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to determine maximum fluorescence (F_m). Actinic light was then applied in eight incremental steps (300–1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$), each lasting 60 s, during which steady-state fluorescence (F_t') and maximum light-adapted fluorescence (F_m') were measured. From these measurements, standard RLC parameters were derived, including maximum relative electron transport rate ($rETR_{\text{max}}$), the initial slope of the curve (α), and the light saturation point (I_k), following established approaches (Baker, 2008; Ralph and Gademann, 2005).

Final harvest metrics: At experiment's end, fresh above- and below-ground biomass were separated and weighed on a precision balance (Ohaus: Scout). The harvested samples were dried in an oven (Carbolite) at 90 °C until constant weight, defined as no further change over a 24 h period. To verify this, a subset of samples (6) was reweighed at intervals of at least 6 h, after which all samples were weighed once the constant-weight criterion was met. Root number was quantified as the count of primary roots emerging directly from the crown, excluding lateral branching. Tiller number was defined as the number of distinct shoots emerging from the base of the plant, each possessing its own leaf whorl. Bud number was recorded as visible axillary buds at the stem base. Leaf number was counted as fully emerged leaves per plant. Ligule (stem) height was measured with a standard metric ruler. Basal diameter was measured at the stem base (approximately 1 cm above the substrate surface) using a digital Vernier caliper.

Germination assessments: Monitored every other day after sowing, days to germination was measured and final percentage germination was taken after fourteen days. Germination was defined as the visible emergence of a green shoot from the seed. Percentage germination (PG) was calculated as: $PG (\%) = (\text{number of germinated seeds} / \text{total number of seeds sown per treatment}) \times 100$.

Data Analysis

Data were analysed by ANOVA using the 'aov' function in base R (R Core Team, 2024), with treatment (PGR), hybrid, and their interaction specified as fixed effects. The analysis focused on assessing treatment responses within each hybrid, and genotypic differences are reported where they inform interpretation of treatment effects. Although the experimental design was blocked, block effects were not included in the model. Preliminary checks showed minimal block variation, so it was excluded from final models. For repeated measurements (like plant height), date was included as an additional factor. Multicollinearity among fixed effects was evaluated using generalized variance inflation factors (GVIFs) computed via the car package in R (Fox & Weisberg, 2019). All predictors (PGR, hybrid, and date) had $GVIF^{(1/(2 \times Df))}$ values below 3.5, indicating acceptable levels of collinearity within the factorial model structure (Zuur *et al.*, 2010). Given the balanced experimental design and the inclusion of all relevant

interactions, these values were considered within a tolerable range and did not compromise model interpretation. Post-hoc pairwise comparisons were conducted using the emmeans package (Lenth, 2023) with Tukey's HSD adjustment for multiple comparisons. Compact letter displays (CLDs) were generated using the multcomp package's 'cld' function (Hothorn *et al.*, 2008) to visualise significant differences among means. All statistical analyses were performed in R, with the level of significance set at $p < 0.05$. Table S1 summarises ANOVA p-values for all traits.

To explore integrated trait responses to plant growth regulator (PGR) treatments across hybrids, a principal component analysis (PCA) was conducted using centred and scaled trait data. Variables included key morphological, biomass, and photosynthetic parameters (stretched height, ligule height, growth rate, tiller count, stem diameter, above- and below-ground biomass, number of roots, $rETR_{max}$, α , and I_k). PCA was performed separately for each hybrid to visualise treatment-driven multivariate shifts while avoiding confounding hybrid effects. Ordination was based on the covariance structure of standardised variables, an approach widely used for exploring coordinated trait responses in plant ecophysiology (Jolliffe & Cadima, 2016; Poorter *et al.*, 2012). Treatment groupings were visualised using 95% confidence ellipses.

RESULTS

Germination Response to Plant Growth Regulators

The effects of plant growth regulators (PGR) on seed germination timing and percentage varied by *Miscanthus* hybrids. In terms of days to germination (Table 1), no significant differences were observed among treatments for GNT3 or GNT43. However, for GNT14, seeds treated with MicroPull germinated significantly later than those in all other treatments ($p < 0.05$), indicating a potential inhibitory effect of this treatment on germination speed.

For percentage germination (Table 1), GNT43 showed no significant differences across all PGR treatments, maintaining consistently high germination rates above 90%. GNT14 exhibited moderate germination percentages (ranging between 53–65%) across treatments, with no statistically significant differences. In contrast, GNT3 displayed a more pronounced response: MicroPull treatment significantly reduced germination

percentage compared to the control and other PGR ($p < 0.05$), suggesting a possible negative effect on seed viability or early seedling development in this hybrid.

Overall, while most PGR did not significantly influence germination dynamics in GNT3 and GNT14, MicroPull had a hybrid-specific inhibitory effect, particularly delaying germination in GNT14 and reducing germination percentage in GNT3.

Table 1. Effects of plant growth regulators on days to germination (DG), percent germination (PG, %), maximum relative electron transport rate ($rETR_{max}$), light saturation point (I_k), and initial quantum yield (α) in *Miscanthus* hybrids.

Hybrid	PGR	DG	PG	$rETR_{max}$	I_k	α
GNT3	Control	10.00 $\pm$ 0.00 ^a	80.60 $\pm$ 4.65 ^a	49.22 $\pm$ 1.59 ^{ab}	149.27 $\pm$ 23.17 ^a	0.34 $\pm$ 0.01 ^d
	IAA	10.29 $\pm$ 0.29 ^a	62.50 $\pm$ 5.59 ^{ab}	48.78 $\pm$ 7.77 ^{ab}	126.00 $\pm$ 13.00 ^{ab}	0.48 $\pm$ 0.04 ^{ab}
	Liquid Ice	10.29 $\pm$ 0.29 ^a	62.50 $\pm$ 6.63 ^{ab}	57.20 $\pm$ 0.68 ^a	134.00 $\pm$ 0.91 ^{ab}	0.43 $\pm$ 0.00 ^{bc}
	MicroPull	10.29 $\pm$ 0.29 ^a	56.90 $\pm$ 6.94 ^b	42.57 $\pm$ 8.25 ^b	94.20 $\pm$ 9.02 ^b	0.51 $\pm$ 0.02 ^a
	NAA	10.58 $\pm$ 0.37 ^a	65.30 $\pm$ 5.01 ^{ab}	51.60 $\pm$ 10.04 ^{ab}	133.00 $\pm$ 11.70 ^{ab}	0.41 $\pm$ 0.01 ^{cd}
GNT14	Control	11.25 $\pm$ 0.38 ^a	59.70 $\pm$ 3.98 ^a	62.12 $\pm$ 7.67 ^b	180.00 $\pm$ 10.10 ^a	0.36 $\pm$ 0.01 ^b
	IAA	10.92 $\pm$ 0.47 ^a	61.10 $\pm$ 4.12 ^a	72.40 $\pm$ 8.23 ^a	199.00 $\pm$ 8.43 ^a	0.37 $\pm$ 0.01 ^b
	Liquid Ice	11.33 $\pm$ 0.42 ^a	63.90 $\pm$ 7.35 ^a	64.66 $\pm$ 2.36 ^{ab}	185.00 $\pm$ 5.97 ^a	0.36 $\pm$ 0.01 ^b
	MicroPull	13.08 $\pm$ 0.72 ^b	52.80 $\pm$ 5.56 ^a	46.95 $\pm$ 13.43 ^c	127.00 $\pm$ 19.90 ^b	0.49 $\pm$ 0.04 ^a
	NAA	11.54 $\pm$ 0.84 ^a	55.60 $\pm$ 4.12 ^a	68.35 $\pm$ 14.84 ^{ab}	210.00 $\pm$ 18.60 ^a	0.36 $\pm$ 0.02 ^b
GNT43	Control	10.00 $\pm$ 0.00 ^a	93.10 $\pm$ 3.98 ^a	29.75 $\pm$ 1.23 ^c	49.20 $\pm$ 1.30 ^c	0.61 $\pm$ 0.01 ^a
	IAA	10.00 $\pm$ 0.00 ^a	97.20 $\pm$ 2.78 ^a	69.68 $\pm$ 4.72 ^a	193.00 $\pm$ 6.61 ^a	0.37 $\pm$ 0.01 ^b
	Liquid Ice	10.00 $\pm$ 0.00 ^a	97.20 $\pm$ 1.72 ^a	68.82 $\pm$ 6.74 ^a	192.00 $\pm$ 8.92 ^a	0.37 $\pm$ 0.01 ^b
	MicroPull	10.00 $\pm$ 0.00 ^a	94.40 $\pm$ 2.78 ^a	53.48 $\pm$ 13.38 ^b	137.00 $\pm$ 14.30 ^b	0.42 $\pm$ 0.01 ^b
	NAA	10.00 $\pm$ 0.00 ^a	93.10 $\pm$ 2.56 ^a	62.22 $\pm$ 7.17 ^{ab}	171.00 $\pm$ 8.10 ^{ab}	0.37 $\pm$ 0.01 ^b

*Letters indicate significant differences among treatments within each hybrid, as determined by Tukey's HSD test ($p < 0.05$). Treatments sharing the same letter within a hybrid and parameter are not statistically different, $n = 6$.

Total Height and Growth Dynamics

The application of PGR noticeably enhanced stem elongation in all three hybrids (Figure 2). Treated seedlings varied from controls soon after the first application and maintained this advantage beyond the cessation of treatment (vertical dashed line). By the final measurement (14th May 2024), Liquid Ice consistently produced the tallest plants, averaging approximately 36 cm in GNT14, 38 cm in GNT3, and 37 cm in GNT43; roughly 1.7–2 $\times$ the height of controls. IAA and NAA treatments also increased final height relative to the control, whereas MicroPull had a more modest effect.

Average daily growth rate (Table 2) mirrored these patterns: Liquid Ice achieved the highest mean rates in GNT14 (0.34 cm day⁻¹) and GNT3 (0.35 cm day⁻¹), both significantly above control (0.21 cm day⁻¹ and 0.20 cm day⁻¹, respectively; $p < 0.05$). In GNT43, Liquid Ice, MicroPull, IAA, and NAA all yielded elevated rates ($\sim$ 0.31–0.36 cm day⁻¹) compared

to control (0.19 cm day^{-1}). MicroPull did not differ significantly from control in any hybrid except in GNT43.

Daily height increment profiles (Figure 3) reveal an early, sharp peak in growth for Liquid Ice and IAA, occurring approximately 3–5 weeks after sowing, followed by a gradual decline until treatment ended. However, IAA and NAA-treated plants exhibited a secondary resurgence in growth rate post-treatment, whereas control and MicroPull treatments maintained a slow but steady increase throughout the experiment.

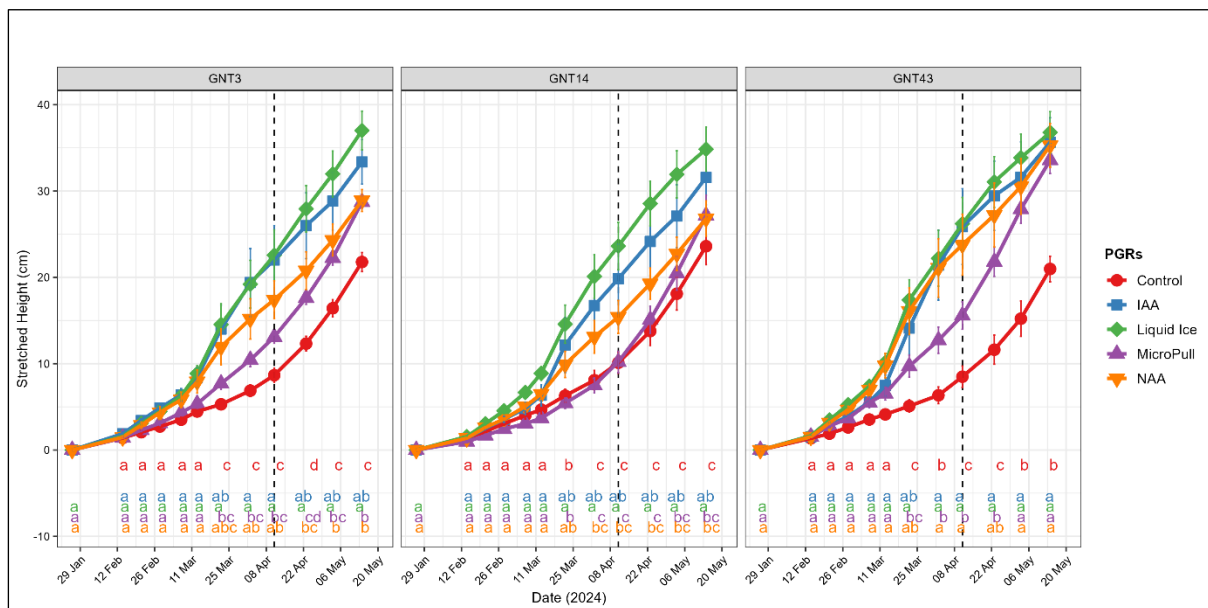


Figure 2. Stretched height of *Miscanthus* plug under PGR treatments. Weekly measurements of stretched height (cm) from 14 February to 14 May 2024 for hybrids GNT14, GNT3, and GNT43. Solid lines represent the period of PGR application, the vertical dashed line marks treatment cessation; data are mean $\pm$ SE ($n = 6$).

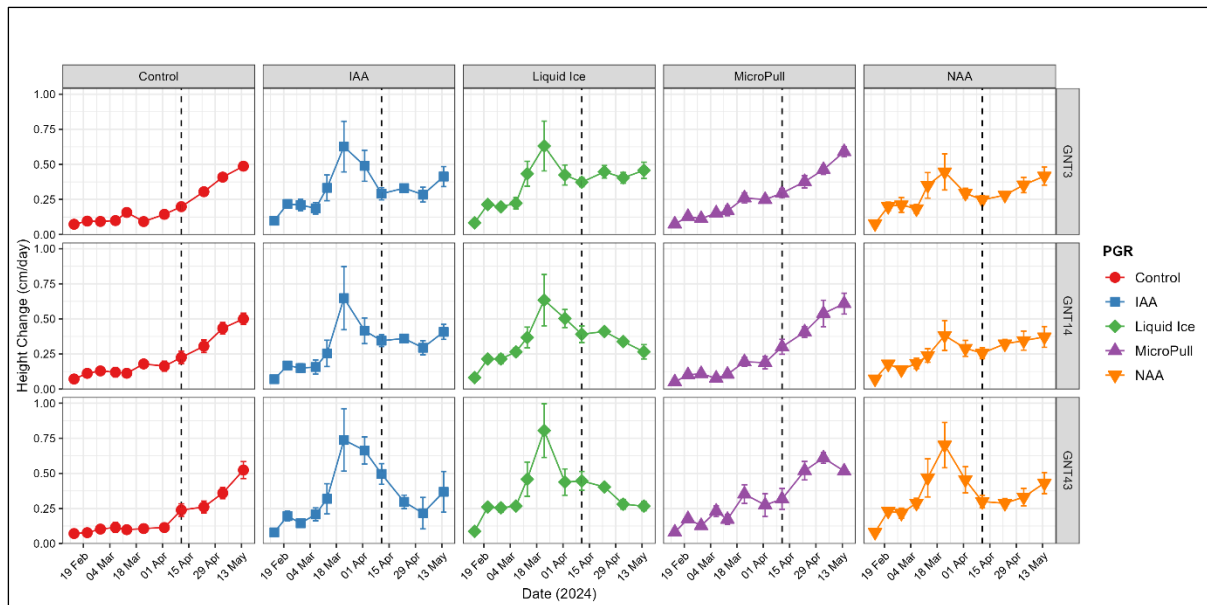


Figure 3. Estimated daily height change profiles for *Miscanthus* plug seedlings. Line plots of mean daily height increment (cm day^{-1}), faceted by hybrid (rows) and PGR treatment (columns). Symbols indicate mean $\pm$ SE ($n = 6$). The vertical dashed line indicates the final PGR application date.

Ligule Height Response to PGR Treatments

Ligule height responded differently to PGR application across the three *Miscanthus* hybrids (Table 2). In GNT43, all four treatments produced a significant increase in ligule height compared to the control ($p < 0.05$), with Liquid Ice stimulating the highest mean height (≈ 12.70 cm), followed closely by IAA, NAA, and MicroPull.

In GNT14, only Liquid Ice resulted in a statistically significant increase in ligule height (≈ 11.00 cm) relative to the control (≈ 6.20 cm; Tukey's HSD, $p < 0.05$). IAA, MicroPull, and NAA treatments did not differ significantly from the control.

In GNT3, Liquid Ice yielded the tallest ligules (≈ 15.40 cm), significantly surpassing the control (≈ 6.93 cm; Tukey's HSD, $p < 0.05$). IAA produced an intermediate ligule height (≈ 12.40 cm), which was also significantly greater than the control but did not differ statistically from the Liquid Ice, MicroPull, or NAA treatments. Both MicroPull and NAA treatments (≈ 8.70 cm and ≈ 9.32 cm, respectively) were not significantly different from the control.

Overall, Liquid Ice consistently enhanced ligule elongation in all hybrids, while IAA showed a significant effect in both GNT3 and GNT43, and a minimal, non-significant trend in GNT14. Other treatments exhibited significant benefits only in GNT43.

Biomass Production and Allocation

Biomass accumulation varied significantly among treatments and across *Miscanthus* hybrids (Figure 4). All treatments resulted in higher biomass than controls, with Liquid Ice consistently producing the greatest increases. In GNT14, Liquid Ice notably increased both above-ground (leaves and stems) and below-ground (roots) biomass. In GNT3, Liquid Ice and IAA treatments significantly enhanced biomass production compared to the control, with both treatments demonstrating similarly strong performance. GNT43 exhibited a notable increase in above-ground biomass with IAA treatment; however, the data showed substantial variability. Liquid Ice, by contrast, reliably enhanced biomass production above controls with lower variability. NAA and MicroPull consistently resulted in moderate biomass increase relative to controls across all hybrids.

Consistent with these patterns, root–shoot ratios (RS, Table 2) showed a clear shift in allocation under Liquid Ice, IAA, and NAA, with all three treatments reducing RS relative to the control in each hybrid. This indicates proportionally greater investment in above-ground structures under these PGR, whereas control and MicroPull treatments maintained higher RS values, reflecting more conservative, root-biased allocation.

Biomass allocation patterns also differed distinctly between treatments (Figure 5). In all three hybrids, control and MicroPull treatments consistently favoured below-ground biomass allocation (roots), accounting for approximately 46–52% of total biomass. Treatments with Liquid Ice, IAA, and NAA shifted allocation toward above-ground structures (leaves and stems). This shift was particularly evident with IAA and Liquid Ice treatments in hybrids GNT3 and GNT43, where stem biomass proportion notably increased at the expense of roots.

In summary, Liquid Ice was the most consistently effective treatment, significantly increasing total biomass production across hybrids and shifting biomass allocation toward above-ground growth. IAA showed strong but variable effects, especially prominent in GNT43. Control and MicroPull treatments exhibited greater root allocation and lower total biomass accumulation, highlighting different physiological growth strategies induced by the tested PGR treatments.

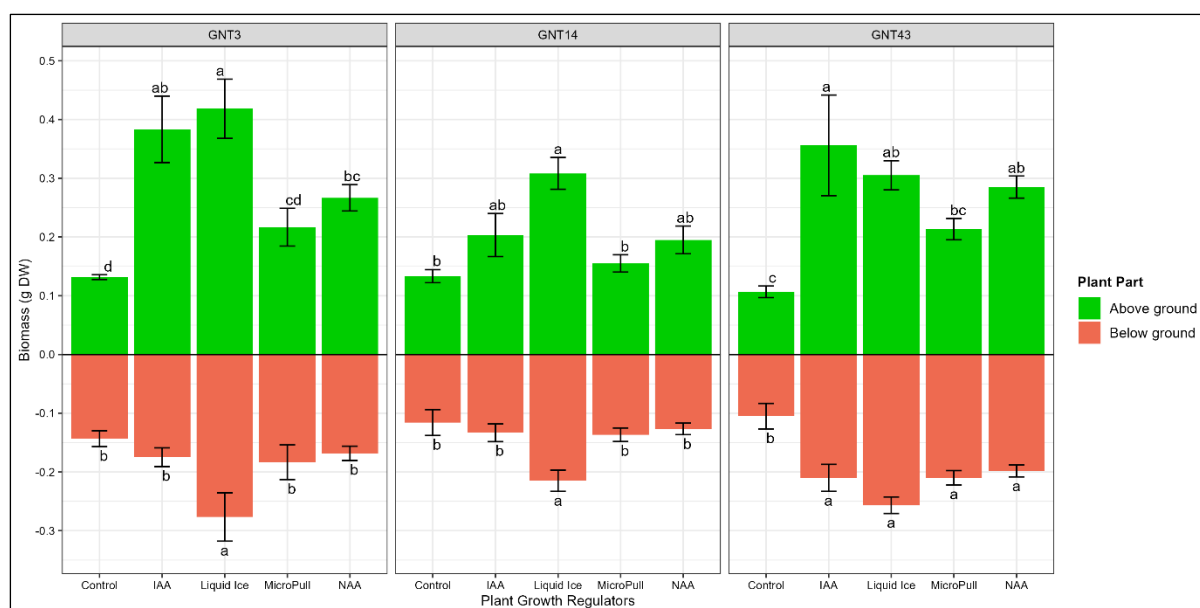


Figure 4. Biomass production (g DW) of *Miscanthus* hybrids (GNT14, GNT3, and GNT43) subjected to different plant growth regulator (PGR) treatments. Coloured bars represent above-ground biomass (leaves and stems; green) and below-ground biomass (roots; brown) $\pm$ SE ($n = 6$). Different letters above error bars indicate significant differences among treatments within each hybrid and plant part (Tukey's HSD, $p < 0.05$).

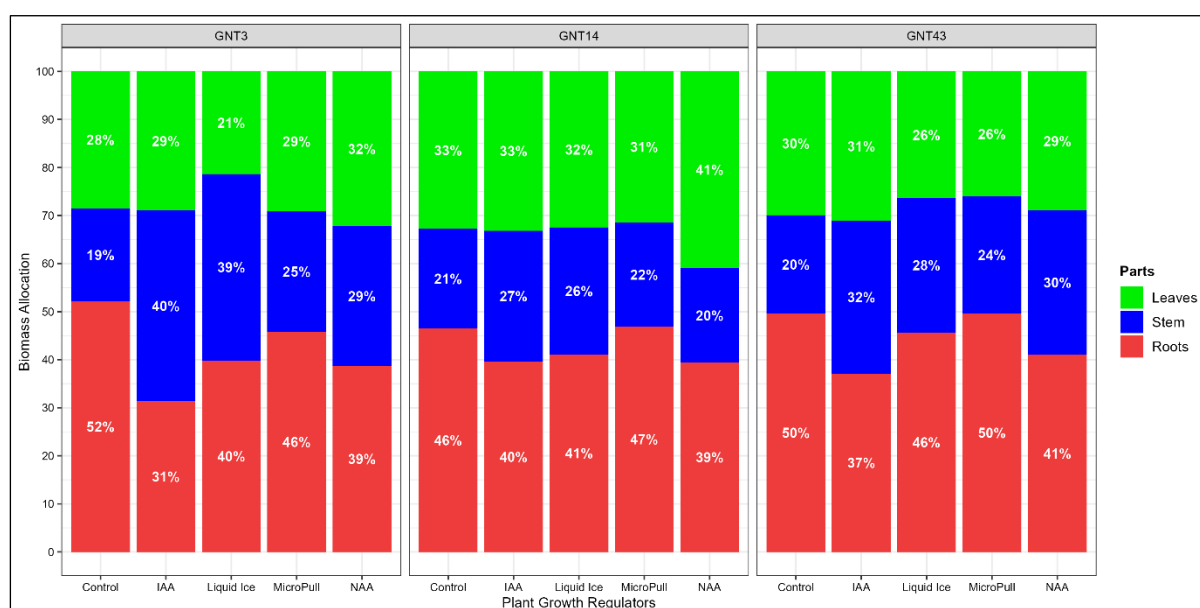


Figure 5. Dry biomass allocation patterns in *Miscanthus* hybrids (GNT14, GNT3, and GNT43) under various PGR treatments. Bars indicate mean biomass distribution (%) among plant components (roots, stems, leaves) for each treatment within hybrids. Percentages reflect the proportional allocation averaged across replicates.

Number of Roots

The response of root number to PGR treatments was hybrid-specific, with significant treatment effects observed in hybrids GNT3 and GNT43, but not in GNT14 (Table 2). In GNT3, Liquid Ice treatment significantly increased the mean number of roots compared to the control and MicroPull treatment. In GNT43, Liquid Ice again resulted in the highest mean root number, significantly exceeding both the control and NAA treatment.

Treatments with IAA and MicroPull also significantly enhanced root production relative to the control in GNT43. In contrast, hybrid GNT14 exhibited no statistically significant variation in root numbers across the treatments.

Tillers and Buds (Basal Axillary Buds)

The response of tiller and bud production to PGR treatments varied among the *Miscanthus* hybrids (Table 2). In GNT43, Liquid Ice and NAA significantly enhanced bud numbers compared to the control, with Liquid Ice yielding the highest mean bud count. No significant differences in tiller numbers were detected among treatments for GNT43. In hybrid GNT14, Liquid Ice significantly increased bud production relative to MicroPull treatment; however, tiller numbers remained unaffected across all treatments. On the other hand, hybrid GNT3 showed no significant treatment-induced differences in either tiller or bud numbers, indicating hybrid-specific responsiveness.

Stem Diameter

Stem diameter responses to plant growth regulator treatments varied notably among the hybrids, with significant treatment effects observed primarily in GNT14 and GNT3 (Table 2). In GNT14, Liquid Ice treatment significantly increased stem diameter compared to IAA but was not significantly different from the control, MicroPull, or NAA treatments. In hybrid GNT3, Liquid Ice again produced the biggest stem diameter, significantly surpassing the control treatment; however, differences between Liquid Ice and the remaining treatments (IAA, MicroPull, NAA) were not statistically significant. Hybrid GNT43 displayed no significant differences among treatments, despite the visibly higher mean diameter values under NAA and Liquid Ice treatments compared to MicroPull and the control. Overall, Liquid Ice consistently enhanced stem diameter, particularly in GNT3 and GNT14 when comparing with IAA, indicating a genotype-dependent response to PGR application.

Table 2. Effects of plant growth regulator treatments on ligule height (LH, cm), growth rate (GR, cm/day), stem diameter (SD, cm²), number of roots (NR), number of tillers (NT), number of buds (NB) and Root:shoot ratio (RS) in Miscanthus hybrids.

Hybrid	PGR	LH	GR	SD	NR	NT	NB	RS
GNT3	Control	6.93 ± 0.97 ^c	0.2 ± 0.01b	1.57 ± 0.09 ^b	6.83 ± 0.95 ^b	1.00 ± 0.00 ^a	0.33 ± 0.14 ^a	1.11 ± 0.15 ^a
	IAA	12.40 ± 1.25 ^{ab}	0.32 ± 0.03a	1.76 ± 0.09 ^{ab}	8.83 ± 0.60 ^{ab}	1.00 ± 0.00 ^a	0.83 ± 0.19 ^a	0.58 ± 0.12 ^b
	Liquid Ice	15.40 ± 1.32 ^a	0.35 ± 0.03a	2.01 ± 0.08 ^a	11.30 ± 1.02 ^a	1.04 ± 0.04 ^a	1.00 ± 0.24 ^a	0.66 ± 0.05 ^b
	MicroPull	8.71 ± 0.27 ^{bc}	0.26 ± 0.01ab	1.61 ± 0.07 ^{ab}	8.00 ± 0.45 ^b	1.06 ± 0.06 ^a	0.60 ± 0.13 ^a	0.84 ± 0.06a ^b
	NAA	9.32 ± 0.58 ^{bc}	0.28 ± 0.02ab	1.70 ± 0.07 ^{ab}	8.50 ± 0.76 ^{ab}	1.00 ± 0.00 ^a	0.79 ± 0.15 ^a	0.66 ± 0.07 ^b
GNT14	Control	6.43 ± 0.69 ^b	0.21 ± 0.02b	1.45 ± 0.09 ^{ab}	6.50 ± 0.43 ^a	1.04 ± 0.04 ^a	0.88 ± 0.28 ^{ab}	0.87 ± 0.09 ^a
	IAA	9.36 ± 1.37 ^{ab}	0.30 ± 0.04ab	1.17 ± 0.04 ^b	6.83 ± 0.40 ^a	1.21 ± 0.16 ^a	1.01 ± 0.21 ^{ab}	0.66 ± 0.08 ^a
	Liquid Ice	11.00 ± 1.10 ^a	0.34 ± 0.03a	1.68 ± 0.10 ^a	7.50 ± 0.56 ^a	1.29 ± 0.21 ^a	1.43 ± 0.12 ^a	0.68 ± 0.07 ^a
	MicroPull	7.07 ± 0.88 ^b	0.24 ± 0.02ab	1.39 ± 0.07 ^{ab}	6.00 ± 0.26 ^a	1.17 ± 0.12 ^a	0.63 ± 0.17 ^b	0.89 ± 0.05 ^a
	NAA	6.86 ± 0.68 ^b	0.25 ± 0.02ab	1.52 ± 0.08 ^{ab}	7.33 ± 0.76 ^a	1.21 ± 0.10 ^a	0.94 ± 0.25 ^{ab}	0.71 ± 0.12 ^a
GNT43	Control	6.20 ± 0.45 ^b	0.19 ± 0.02b	1.42 ± 0.22 ^a	5.00 ± 0.73 ^c	1.00 ± 0.00 ^a	0.58 ± 0.24 ^c	1.03 ± 0.18 ^a
	IAA	11.70 ± 1.00 ^a	0.34 ± 0.03a	1.53 ± 0.17 ^a	8.67 ± 1.23 ^{ab}	1.00 ± 0.00 ^a	1.04 ± 0.18 ^{bc}	0.75 ± 0.16 ^a
	Liquid Ice	12.70 ± 0.98 ^a	0.36 ± 0.03a	1.74 ± 0.08 ^a	10.8 ± 0.60 ^a	1.10 ± 0.06 ^a	2.00 ± 0.00 ^a	0.87 ± 0.06 ^a
	MicroPull	10.4 ± 0.68 ^a	0.31 ± 0.02a	1.36 ± 0.13 ^a	8.17 ± 0.91 ^{ab}	1.18 ± 0.09 ^a	1.03 ± 0.28 ^{bc}	0.95 ± 0.11 ^a
	NAA	12.4 ± 1.32 ^a	0.34 ± 0.03a	1.75 ± 0.11 ^a	7.00 ± 0.68 ^{bc}	1.06 ± 0.06 ^a	1.67 ± 0.22 ^{ab}	0.74 ± 0.07 ^a

*Letters indicate significant differences among treatments within each hybrid, as determined by Tukey's HSD test ($p < 0.05$).
Treatments sharing the same letter within a hybrid are not statistically different, $n = 6$.

Photosynthetic Parameters

Photosynthetic responses to PGR treatments varied notably across the hybrids, with genotype-dependent patterns observed for maximum relative electron transport rate ($rETR_{max}$), initial quantum yield (α), and light saturation point (I_k).

For maximum relative electron transport rate ($rETR_{max}$, Table 1), IAA produced the highest $rETR_{max}$ values in GNT14, significantly exceeding both control and MicroPull treatments. In GNT3, Liquid Ice treatment significantly elevated $rETR_{max}$ compared to MicroPull but was not significantly different from other treatments. Similarly, GNT43 plants treated with IAA and Liquid Ice showed significantly higher $rETR_{max}$ values than those treated with MicroPull or control. MicroPull consistently exhibited the lowest $rETR_{max}$ values across all hybrids.

For the initial slope of the light curve (α , Table 1), MicroPull treatment resulted in the highest α values in GNT14 and GNT3, significantly exceeding all other treatments. In

contrast, GNT43 exhibited a distinct response, with control plants having the highest α value, significantly greater than all other treatments.

The light saturation point (I_k , Table 1) was significantly affected by the treatments in a hybrid-specific manner. In GNT14, MicroPull significantly decreased I_k compared to all other treatments. For GNT3, MicroPull similarly resulted in significantly lower I_k values than the control, with intermediate responses from other treatments. In GNT43, Liquid Ice and IAA treatments produced the highest I_k values, significantly greater than those of the control and MicroPull.

Overall, these results illustrate complex and hybrid-specific photosynthetic responses, highlighting IAA and Liquid Ice as consistently effective at enhancing photosynthetic performance ($rETR_{max}$ and I_k), while MicroPull influenced initial quantum yield (α) most notably in GNT14 and GNT3.

Principal Component Analysis

Principal component analysis (PCA) indicated moderate, hybrid-dependent multivariate responses to PGR treatments (Figure 6), with PC1 (38%) associated primarily with plant size, biomass accumulation, and photosynthetic capacity, and PC2 (17.4%) reflecting variation in photosynthetic efficiency and allocation-related traits. In GNT3, Liquid Ice showed partial separation from the control along PC1, while IAA, NAA, and MicroPull exhibited considerable overlap. In GNT14, treatment groups largely overlapped, with only a slight shift of Liquid Ice towards higher PC1 values. In GNT43, clearer separation of the control from treated plants was observed along PC1, although overlap among PGR treatments remained. Overall, PCA revealed only limited separation among treatments, indicating that multivariate responses were modest and strongly genotype dependent.

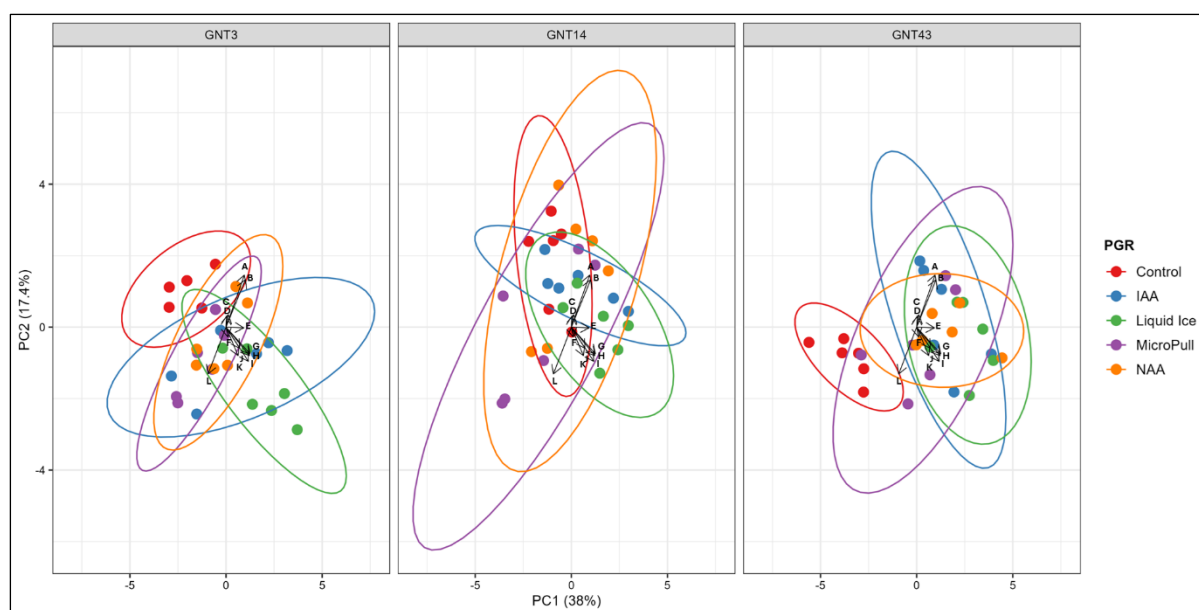


Figure 6. Principal component analysis (PCA) of morphological, biomass, and photosynthetic traits for *Miscanthus* hybrids under plant growth regulator treatments. Points represent individual replicates; ellipses indicate confidence intervals. Trait loadings are shown as vectors (A: ik, B: etr_max, C: above ground biomass, D: below ground biomass, E: Buds, F: Tillers, G: growth rate, H: stretched height, I: ligule height, J: stem diameter, K: number of roots, L: alpha).

DISCUSSION

This study demonstrates that PGR treatments applied during the plug stage influence early development in *Miscanthus* hybrids. These effects vary among hybrids and extend to biomass accumulation and photosynthetic function. Among the treatments evaluated, Liquid Ice and IAA were the most effective, promoting significant increases in plant height, total biomass, stem girth, ligule length, and photosynthetic performance. In contrast, MicroPull exhibited hybrid-specific effects, occasionally suppressing germination or limiting growth responses.

Germination and Early Establishment

MicroPull had been observed to delay germination in GNT14 and reduced germination percentage in GNT3 (Table 1) underscoring the complex and hybrid-dependent nature of its response during the earliest establishment phase. In contrast, auxin-based treatments (IAA, NAA) and Liquid Ice did not negatively affect germination dynamics in any hybrid, supporting evidence from other crop systems that carefully selected or dosed auxins can be well-matched with high establishment rates (Zhao *et al.*, 2020; El-Mergawi *et al.*, 2020; Ellouzi *et al.*, 2024). The lack of effect in GNT43 suggests some hybrids may be intrinsically less sensitive to either the inhibitory or promotive aspects of these compounds during seedling emergence. Such findings highlight the importance of hybrid

selection when designing PGR protocols for *Miscanthus* plug production. It is equally possible that different dosage is required to command any effect.

Root and Axillary Development (Number of Roots, Tillers, and Buds)

Root and axillary organ development demonstrated hybrid-specific sensitivity to PGR treatments. In GNT3 and GNT43, Liquid Ice substantially increased the number of main roots compared to the control (1.7 and 2-fold increase, respectively), with IAA and MicroPull also enhancing root proliferation in GNT43 (Table 2). In GNT14, no treatment effects were detected. Tiller production was generally unresponsive to PGR treatments (Table 2), indicating that early tillering may be more genetically constrained under plug conditions. However, bud number showed selective responsiveness; in GNT43, both Liquid Ice and NAA significantly increased bud production relative to the control, and in GNT14, Liquid Ice increased bud count compared to MicroPull. These results suggest that while some PGR can promote root and bud initiation in a hybrid-specific manner, tillering is less plastic at the plug stage. Enhanced root and bud formation may underpin improved plug vigor and later field establishment, as documented in other studies (Williamson *et al.*, 2012; Tang *et al.*, 2024).

Vegetative Growth and Biomass Allocation

The pronounced enhancement of stretched plant height by Liquid Ice and IAA across all hybrids (Figure 2) is likely attributable to auxin-mediated stimulation of cell division and expansion, as well as improved nutrient assimilation associated with biostimulant action (Fei *et al.*, 2020). Notably, these treatments not only increase total biomass by up to 2.5-fold but also shifted allocation toward above-ground organs (Figure 4 and 5). While increased shoot biomass is advantageous for eventual bioenergy yield (Heaton *et al.*, 2008), its role during early establishment remains complex, as perennial grasses often prioritise below-ground development to support persistence. However, greater early shoot growth may enhance carbon assimilation and thereby increase assimilate availability for subsequent root and rhizome development, as suggested by coordinated biomass allocation frameworks (Poorter *et al.*, 2012; Reich, 2014). In contrast, the greater root allocation observed in MicroPull, and control treatments (Figure 5) may enhance early establishment and stress resilience, indicating a trade-off between above-ground growth and below-ground investment. Similar PGR-driven shifts in allocation have been

documented in studies in other plants, reinforcing the broader relevance of these findings (Severino *et al.*, 2024).

Other Structural and Morphological Traits

Structural parameters such as ligule height and stem diameter (Table 2) also exhibited strong, hybrid-dependent responses to PGR application. Liquid Ice consistently promoted the greatest ligule elongation and stem thickening in GNT14 and GNT3, likely reflecting enhanced vascular differentiation and cell wall development under elevated auxin conditions (Lavania *et al.*, 2021). The limited effect in GNT43 may indicate either a genetic ceiling for structural response or alternative regulatory pathways in this hybrid, meriting further anatomical and molecular characterization.

Photosynthetic Function

PGR treatments exerted significant effects on photosynthetic parameters, though responses were highly hybrid – specific. IAA and Liquid Ice enhanced maximum relative electron transport rates ($rETR_{max}$) and light saturation points (I_k) in all hybrids (Table 1), consistent with improved photosynthetic capacity and acclimation to higher light regimes (Urbutis *et al.*, 2023). Interestingly, MicroPull enhanced initial quantum yield (α) in GNT14 and GNT3 (Table 2), yet suppressed I_k , suggesting a trade-off between efficiency at low light and capacity at high irradiance. These patterns indicate that PGR may modulate both the biochemical and biophysical limitations of photosynthesis, potentially via effects on chloroplast development or pigment-protein complex assembly (Vercruyssen *et al.*, 2015; Müller & Munné-Bosch, 2021). Understanding the mechanistic basis for these hybrid-specific photosynthetic shifts could inform more nuanced deployment of PGR in plug propagation.

Multivariate Trait Responses to PGR Treatments

The PCA provides a useful but exploratory perspective on how PGR treatments influence multiple traits simultaneously in *Miscanthus* plugs. Although some directional shifts along PC1 were observed, particularly for Liquid Ice in certain hybrids (like GNT43), the degree of separation among treatments was generally modest and accompanied by substantial overlap. This suggests that while PGR may influence coordinated trait

responses related to plant size, biomass accumulation, and photosynthetic capacity, these effects are not consistently distinct across all hybrids.

MicroPull showed a tendency to associate with traits linked to photosynthetic efficiency (α) rather than structural growth; however, this pattern was not strongly separated from other treatments, indicating only a partial decoupling between efficiency and biomass-related traits. Similar patterns have been reported under conditions where physiological adjustments do not directly translate into increased growth (Müller & Munné-Bosch, 2021).

Overall, the PCA supports the conclusion that responses to PGR treatments are strongly genotype-dependent but also highlights the complexity and variability of these responses. Given that the first two components explained a moderate proportion of total variance, these multivariate patterns should be interpreted cautiously and alongside univariate trait analyses. The results nonetheless reinforce the need for hybrid-specific optimisation of PGR application strategies, particularly when developing scalable plug-based propagation systems under variable environmental conditions (Clifton-Brown *et al.*, 2017; Ashman *et al.*, 2023b).

Leveraging PGR-Induced Morphology for Better *Miscanthus* Stand Establishment and Yield Potential

The slight delay in early shoot emergence observed under MicroPull or NAA treatments may have implications for the plug-to-field transition phase rather than initial germination under controlled conditions. In plug-based systems, seedlings are transplanted into variable field environments, where synchronisation of active growth with favourable conditions can influence establishment success. A moderated delay in shoot expansion may reduce exposure of metabolically active tissues to transient stress events immediately post-transplant, although this remains speculative. Timing of emergence and early growth has been shown to influence establishment outcomes in *Miscanthus* under field conditions (Clifton-Brown *et al.*, 2017), but the extent to which plug-stage treatments translate to these dynamics requires further validation.

Furthermore, improved plant height in the first year, particularly following treatments like Liquid Ice and IAA, can promote faster canopy closure and light interception, which helps

suppress weed growth and reduces reliance on herbicides. Lewandowski *et al.* (2016) highlighted that taller *Miscanthus* seedlings would gain competitive advantage by rapidly overtopping competing vegetation. This response aligns with the broader concept of shade avoidance, where vertical growth enables competitive light capture, a common strategy across many plant species (Gruntman *et al.*, 2017; Gautrat *et al.*, 2025). Similarly, Ashman *et al.* (2023a) emphasized the importance of early morphological vigour, noting that traits such as increased ligule height and faster vertical growth contribute to effective weed suppression in seed-propagated *Miscanthus* systems.

Biomass allocation, particularly the distribution of assimilates between shoots and roots, is a key determinant of overwinter survival and regrowth in perennial bioenergy crops such as *Miscanthus*. While these processes are realised in established stands, allocation patterns are initiated during early developmental stages, including the plug phase. Treatments in this study altered biomass partitioning at this stage (plug phase), suggesting potential implications for subsequent root reserve formation and persistence after transplanting. For example, greater root development has been associated with increased non-structural carbohydrate storage and improved spring emergence (Heaton *et al.*, 2008). However, the extent to which these early shifts translate into field performance remains to be determined.

Also, the morphological changes induced by PGR treatments provide practical opportunities for optimizing *Miscanthus* plug production. By modulating traits like stretched height, ligule elongation, or basal girth, it becomes feasible to tailor plugs to site-specific challenges, such as maximizing early height for weed competition or thicker stems for better handling in mechanical planters. Studies by Ashman *et al.* (2023a) demonstrated that seed plug establishment protocols can benefit from deliberate manipulation of plug morphology to enhance field performance, while Zheng *et al.* (2021) highlighted the need for plug customization based on planting conditions to minimize establishment failures.

Alterations in photosynthetic parameters, such as increased $rETR_{max}$ and I_k , observed in response to certain PGR treatments may provide seedlings with a competitive advantage by enhancing carbon assimilation and increasing energy reserves necessary for rhizome

development. Baker (2008) demonstrated that elevated photosynthetic capacity improves carbohydrate availability for storage, thereby strengthening resilience and promoting regrowth in perennial species. Similarly, Lee *et al.* (2021) emphasized that maximizing the energy potential of bioenergy grasses requires improving photosynthetic efficiency, particularly under fluctuating light conditions, which are common in field environments.

From a production perspective, these findings have important cost implications. Extending the plug phase to achieve larger, more physiologically developed seedlings can improve establishment success, but this comes with increased greenhouse costs, particularly energy, labour, and inputs (Clifton-Brown *et al.*, 2017; Hastings *et al.*, 2017). The enhanced growth and biomass accumulation observed under Liquid Ice and IAA treatments suggest that PGR application may partially offset this trade-off by accelerating seedling development within shorter production windows. Such gains could reduce time-to-transplant without compromising plant quality, improving overall system efficiency.

Finally, Hybrid-specific responses and practical implications further underscore the complexity of optimizing *Miscanthus* establishment through PGR treatments. The strong interactions between PGR treatments and hybrid background observed in this study emphasize that uniform protocols are unlikely to maximize performance across different *Miscanthus* germplasm. Future research should systematically characterise dose-response relationships, extend trials to post-transplant performance in field settings, and investigate underlying physiological and molecular markers of PGR sensitivity. Integrating plant growth regulator applications with complementary plug optimisation strategies e.g. fine-tuned light, temperature, or substrate management, may result in additive or even synergistic improvements in crop establishment and yield. While these interventions can introduce additional production costs, they may prove essential in regions where conventional practices have led to poor establishment, offering a critical tool for adapting to and mitigating the impacts of a changing climate. Ultimately, developing hybrid-tailored PGR regimes holds promise for advancing the scalability and resilience of seed-based *Miscanthus* bioenergy cropping systems, thereby contributing to the broader goals of sustainable biomass production and climate mitigation.

CONCLUSION

This research provides robust evidence that targeted application of plant growth regulators or biostimulants during the plug stage can significantly enhance early development, resource allocation, and photosynthetic function of *Miscanthus* hybrids, with pronounced hybrid-dependent effects. Liquid Ice and IAA consistently promoted greater seedling vigour, increased above-ground biomass, and improved key physiological traits, while MicroPull and NAA exhibited more variable or selective effects. Importantly, the findings highlight that PGR efficacy is not uniform across hybrids, underscoring the need for hybrid-specific management strategies in *Miscanthus* plug production systems.

The outcomes of this study have direct implications for the optimisation of seed-based propagation in *Miscanthus*, a crop of strategic importance for sustainable bioenergy. By identifying effective PGR treatments and clarifying their impacts on early plant performance, this work lays the groundwork for more scalable and reliable plug production protocols. These improvements in early vigour and biomass allocation may be particularly relevant for establishing *Miscanthus* on marginal or stress-prone lands, where successful crop establishment remains a key constraint. Future research should extend these findings to field conditions and explore the mechanistic basis for hybrid-specific PGR responses. Ultimately, integrating optimised PGR regimes with other plug production enhancements will accelerate the deployment of *Miscanthus* as a resilient, high-yielding bioenergy crop, advancing global efforts toward renewable energy and climate mitigation.

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Supplementary materials

Supplementary Table S1. Summary of factorial ANOVA p-values for treatment (PGR), hybrid, and their interaction across all measured morphological, biomass, and photosynthetic traits.

Traits	PGR	Hybrid	PGR:Hybrid	Date
Days to germination	0.1263	<0.001	0.1095	
Percentage germination	0.0431	<0.001	0.2102	
Relative electron transport rate (rETR _{max})	<0.001	<0.001	<0.001	
Light saturation point (I _k)	<0.001	<0.001	<0.001	
Initial quantum yield (α)	<0.001	<0.001	<0.001	
Stretched height	<0.001	<0.001	<0.001	<0.001
Growth rate	<0.001	0.0340	0.5099	
Ligule height	<0.001	<0.001	0.0401	
Above-ground biomass	<0.001	0.001	0.0923	
Below-ground biomass	<0.001	<0.001	0.3569	
Root:Shoot ratio	0.001	0.2234	0.8114	
Stem diameter	<0.001	<0.001	0.3532	
Number of roots	<0.001	0.001	0.0651	
Number of tillers	0.3798	0.0133	0.9063	
Number of buds	<0.001	<0.001	0.1855	

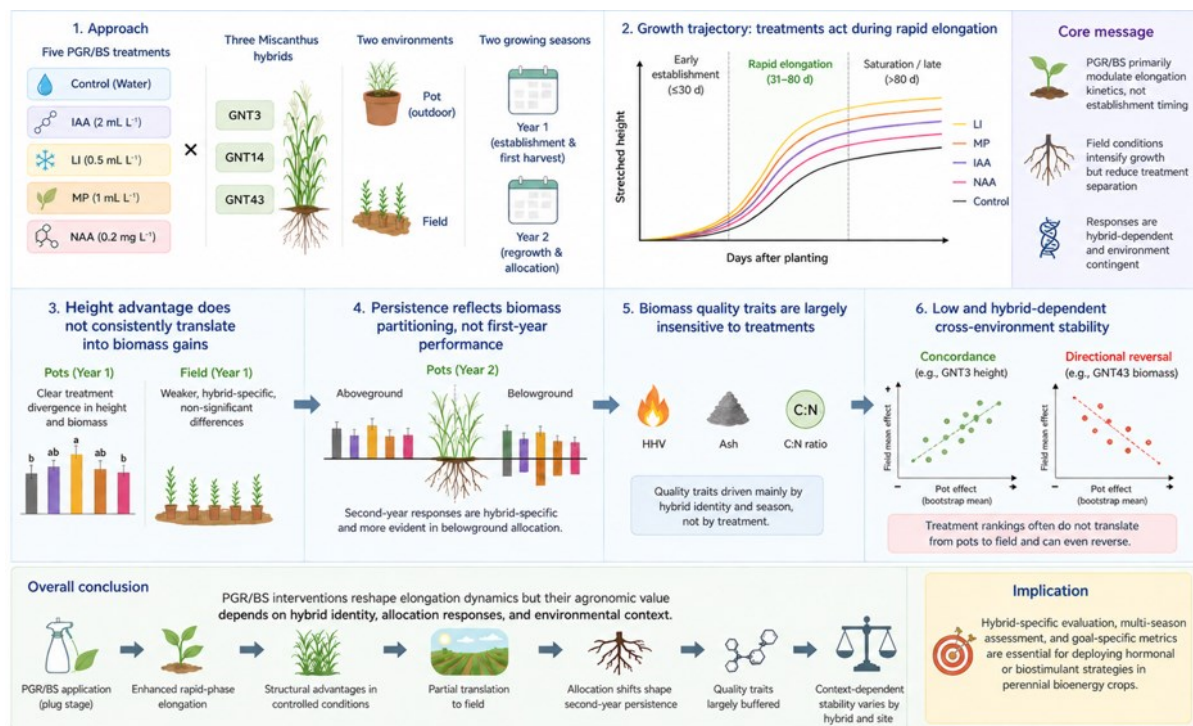
Supplementary Table S2. Germination percentage (%G), seedling height (H), and selection outcomes for all biostimulant and phytohormone treatments tested at full and half dose on GNT3 during the preliminary screening experiment.

Treatment (concentration)	Type	Company	Dose	%G	H (cm)	Selected ?
Water	Control	-	-	90	6.77 ± 0.77	-
Ceres Micropull	Biostimulant	Ceres Biotics	Full	90	-	No
Ceres Micropull	Biostimulant	Ceres Biotics	Half	93	11.75 ± 1.28	Yes
Elixir	Biostimulant	Evoponic	Full	96	9.27 ± 0.93	No
Elixir	Biostimulant	Evoponic	Half	91	-	No
Ethrel C (Ethephon)	Phytohormone	Philip Harris	Full	20	-	No
Ethrel C (Ethephon)	Phytohormone	Philip Harris	Half	86	-	No
Gibberellic acid	Phytohormone	Philip Harris	Full	98	7.10 ± 0.71	No
Gibberellic acid	Phytohormone	Philip Harris	Half	93	-	No
Indole-3-acetic acid	Phytohormone	The Plant Growth Company	Full	95	-	No
Indole-3-acetic acid	Phytohormone	The Plant Growth Company	Half	95	15.00 ± 1.66	Yes
Indole-3-butyric acid	Phytohormone	The Plant Growth Company	Half	89	-	No
Indole-3-butyric acid	Phytohormone	The Plant Growth Company	Full	90	-	No
Liquid ice	Biostimulant	Evoponic	Full	93	16.28 ± 1.61	Yes
Liquid ice	Biostimulant	Evoponic	Half	90	-	No
Naphthalene acetic acid	Phytohormone	Philip Harris	Full	98	-	No
Naphthalene acetic acid	Phytohormone	Philip Harris	Half	98	15.01 ± 1.53	Yes
Super rootz	Biostimulant	Evoponic	Half	93	7.77 ± 0.81	No
Super rootz	Biostimulant	Evoponic	Full	84	-	No
Canna Rhizotonic	Biostimulant	Canna	Full	87	-	No
Canna Rhizotonic	Biostimulant	Canna	Half	90	-	No
Mammoth P	Biostimulant	Mammoth ag	Full	89	-	No
Mammoth P	Biostimulant	Mammoth ag	Half	90	-	No

Germination percentage (%G) was assessed 7 days after sowing, with 100 seeds sown per treatment. A subset of seedlings was then transferred individually into celled trays, and plant height was recorded after 6 weeks. The preliminary screening was conducted using hybrid GNT3, which was selected because its germination and early growth performance was intermediate among the three hybrids—stronger than GNT14 but not exceeding GNT43—making it a representative genotype for initial treatment evaluation. Treatments were transferred only when germination percentage exceeded that of the control; where full- and half-dose treatments produced similar germination outcomes, the half-dose treatment was chosen.

Chapter 4

How Growth Regulators Reshape *Miscanthus*: Hybrid-Specific Biomass Allocation and Cross-Environment Stability



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ABSTRACT

Plant growth regulators (PGR) and biostimulants (BS) offer a potential approach for modifying early structural development in *Miscanthus*, but whether treatment-induced growth responses persist across hybrids, environments and growing seasons remains uncertain. Five PGR/BS treatments were evaluated across three seed-derived *Miscanthus* hybrids in a pot experiment conducted over two growing seasons and a field experiment conducted during the second growing season. Growth trajectories, biomass accumulation and allocation, and biomass quality were assessed to determine whether treatments primarily affected rapid growth phase, translated into biomass gains, and influenced subsequent biomass partitioning. Growth followed a sigmoidal trajectory, with treatment effects expressed predominantly during rapid elongation phase rather than establishment. Treatment responses were hybrid-dependent, with particularly strong height responses to IAA, Liquid Ice and MicroPull in field-grown GNT3, although these structural differences did not result in significant biomass increases. Second-year pot responses differed from first-year structural responses, with treatment effects on biomass allocation varying among hybrids and, in GNT43, being more evident belowground. Biomass quality traits were comparatively insensitive to treatment, with variation in HHV, ash content and C:N ratio driven primarily by hybrid and seasonal or environmental effects. Cross-environment analysis showed strong differences in endpoint height and biomass between pot and field experiments, together with hybrid- and environment-dependent treatment responses. Overall, PGR/BS treatments primarily modified rapid growth phase, but greater structural growth did not consistently translate into biomass gain or subsequent persistence, highlighting the importance of hybrid-specific evaluation and field validation when assessing growth-modulating strategies for perennial bioenergy crops.

Keywords: *Miscanthus*; plant growth regulators; biostimulants; rapid growth phase; biomass allocation; hybrid-dependent responses; cross-environment responses

INTRODUCTION

Decarbonising energy systems requires biomass feedstocks that combine high yield potential with low agronomic inputs and stable performance across heterogeneous environments (Lewandowski *et al.*, 2000; Heaton *et al.*, 2008; Clifton-Brown *et al.*, 2017). Perennial C₄ grasses such as *Miscanthus* are leading candidates for temperate bioenergy systems because of their high radiation-use efficiency, extensive belowground storage, and sustained productivity on marginal land (Clifton-Brown *et al.*, 2001; Lewandowski *et al.*, 2003; Hastings *et al.*, 2009). However, translating genetic yield potential into consistent field performance remains a major bottleneck. Genotype by environment (G×E) interactions strongly shape canopy development, seasonal biomass accumulation, and multi-year persistence in perennial plants including *Miscanthus* (Clifton-Brown *et al.*, 2004; van Eeuwijk *et al.*, 2016). Early developmental dynamics are particularly significant because variation in establishment and rapid elongation phases can influence seasonal growth and carbon allocation and may contribute to subsequent rhizome development and long-term productivity. However, established perennial plants can also compensate for early differences through subsequent canopy development and resource allocation, meaning that the extent to which early-season responses persist into long-term biomass production is context-dependent.

Plant growth regulators (PGR) and biostimulants (BS) represent a promising yet underexplored approach for modulating early structural development in perennial bioenergy crops. Exogenous hormonal inputs in plants generally influence elongation kinetics, branching patterns (or tillering in the case of grasses like *Miscanthus*), and carbon partitioning through interactions with endogenous signalling networks (Santner & Estelle, 2009). In annual crops, such manipulation can reshape canopy architecture and yield components. In perennial systems, however, hormonal modulation may have deeper implications, affecting rhizome carbohydrate storage, overwinter survival, and regrowth capacity (Wolters & Jürgens, 2009; Depuydt & Hardtke, 2011). Because *Miscanthus* productivity depends critically on reserve accumulation and seasonal carbon allocation (Clifton-Brown & Lewandowski, 2000; Strullu *et al.*, 2011), early structural manipulation may generate delayed or carryover effects not detectable in

first-year biomass alone. Whether PGR/BS-induced changes in early structural growth persist into subsequent biomass production and allocation remains unresolved.

In the reviewed literature, evaluations of PGR and BS efficacy were predominantly conducted under controlled-environment or pot-based conditions (Obaje *et al.*, *under review*, Chapter 2). Controlled systems reduce environmental variation and can facilitate the detection of treatment effects (Passioura, 2006), but they also constrain rooting volume and homogenise resource availability (Poorter *et al.*, 2012; 2016). Such differences in growing conditions may alter allocation responses relative to field environments, potentially exaggerating or masking treatment responsiveness. Consequently, treatment responses observed under controlled conditions may not translate directly to field performance. Because hybrids can differ in hormonal sensitivity and resource-response thresholds, variation in treatment response between controlled and field environments is biologically plausible and agronomically relevant (Obaje *et al.*, 2026). Yet few studies explicitly examine the consistency of PGR or BS responses across controlled and field environments, particularly in perennial bioenergy crops.

Evaluating treatment responses across contrasting environments therefore requires consideration of both the magnitude and consistency of growth responses. Differences in environmental conditions can alter the expression of treatment effects, while hybrid-specific responses may further complicate the transfer of results from controlled systems to field conditions. In *Miscanthus*, this is particularly relevant because early structural growth, seasonal biomass accumulation, and subsequent biomass allocation are not necessarily coupled. Examining growth trajectories alongside biomass responses across environments and growing seasons can therefore help determine whether early treatment-induced differences are maintained, compensated for, or redistributed over time.

Growth in *Miscanthus* follows a trajectory characterised by early establishment, a rapid elongation phase, and late-season saturation (Clifton-Brown *et al.*, 2001). During rapid growth phase of plants, biomass accumulation is strongly influenced by plant size and growth rate, such that variation in elongation can influence subsequent canopy development and seasonal biomass accumulation (Poorter *et al.*, 2012). Empirical

evidence indicates that early-season stem elongation plays an important role in determining final biomass in *Miscanthus*, although interactions with later structural constraints can limit seasonal growth (Robson *et al.*, 2025). Because this rapid phase involves processes that are responsive to hormonal regulation, exogenous interventions may influence growth primarily by altering elongation dynamics rather than establishment timing. Whether such early structural modification consistently translates into biomass gain, multi-season persistence, or changes in biomass composition, however, remains unresolved.

Here, this study evaluates multiple PGR and BS treatments across three *Miscanthus* hybrids in pot and field environments over two growing seasons. The study tests whether PGR and BS application primarily modulates rapid growth phase rather than establishment timing; whether structural differences observed in controlled conditions translate into field biomass responses; whether early hormonal modulation influences second-year performance through changes in biomass allocation; and whether treatment responses differ among hybrids and between environments. By integrating dynamic growth-phase analysis with measurements of biomass and biomass quality across contrasting environments and growing seasons, the study examines how early treatment-induced changes in plant structure are expressed in subsequent productivity and allocation. Understanding whether treatments enhance early growth and whether these responses persist across environments and seasons is important for evaluating the potential of PGR and BS approaches in perennial bioenergy production.

MATERIALS AND METHODS

Experimental overview

Two coordinated experiments were conducted to evaluate structural development, biomass accumulation, compositional traits, and treatment responses across contrasting growing environments in three *Miscanthus* hybrids. A pot experiment was performed outdoors over two consecutive growing seasons to assess first-year growth and second-year persistence. A parallel field experiment was established during the second growing season using a separate set of planting material. The same treatment

regimes were applied to these plants during Year 2, but they were independent of the planting material used in the Year 1 experiment.

Both experiments utilised the same hybrids and PGR/BS treatments, enabling comparison of treatment responses across environments. The design allowed assessment of growth-phase modulation, biomass accumulation and allocation, and biomass quality traits across contrasting growing conditions.

Plant materials, PGR/BS treatments and nursery environment

Three commercially relevant seed-derived *Miscanthus* hybrids (GNT3 - Artemis, GNT14 - Brontes, and GNT43 - Aphrodite) originating from the Giant-Link *Miscanthus* breeding programme were used. Seed material was supplied by Terravesta Ltd. (UK). Further details on these hybrids are provided in Obaje *et al.* (2026); Chapter 3.

Seeds were germinated in a controlled glasshouse at Liverpool John Moores University (53°24'45.1"N, 2°58'47.7"W). Glasshouse conditions, temperature and humidity averaged approximately 20 ± 5 °C and 50 ± 10 %, respectively. A 16:8 h light:dark photoperiod was maintained using supplementary Kroptek KP4 broad-spectrum LED lighting (Kroptek, London, UK) during winter months. Seeds were sown on 26th January 2024 into Levington Multipurpose Compost (Evergreen Garden Care, UK) in 12-cell propagation trays ([Mixceco](#)). To maintain a high-humidity environment during germination, trays were fitted with their accompanying transparent lids for the initial two weeks following sowing.

Five treatments were evaluated: Water (control), Indole-3-acetic acid (IAA), 1-Naphthaleneacetic acid (NAA), Liquid Ice (LI), and MicroPull (MP) (Table 1). Treatments were applied via base-watering at the plug stage by delivering solution (approximately 250 mL) into trays beneath the cells. Applications were conducted weekly during the first six weeks of growth and subsequently twice weekly as water demand increased. PGR/BS treatments were maintained for eight weeks, followed by a two-week hardening-off period during which only water was applied across all treatments. No additional PGR/BS was applied following transplanting. Treatment concentrations (Table 1) were selected based on manufacturer recommendations and preliminary dose–response screening conducted in a parallel experiment (Chapter 3; Obaje *et al.*, 2026).

Table 1. Summary of plant growth regulator (PGR) and Biostimulant (BS) treatments applied during the plug stage, including concentrations and suppliers. All treatments were delivered via base-watering during the first eight weeks of growth. Water (control), Indole-3-acetic acid (IAA), 1-Naphthaleneacetic acid (NAA), Liquid Ice (LI), and MicroPull (MP)

Treatment	Control	LI	MP	IAA	NAA
Concentration	-	0.5 mL L ⁻¹	1 mL L ⁻¹	2 mL L ⁻¹	0.2 mg L ⁻¹
Supplier	-	Evoponic	Ceres Biotics	The Plant Growth Company	Philip Harris

Pot experiment (outdoor conditions)

Following completion of the glasshouse phase, plugs were cut back to 3 cm to standardise initial aboveground biomass (similar to commercial production protocol) and transplanted into 5 L pots (Muddy Hands Ltd., Birmingham, UK) filled with commercial topsoil (Wickes Ltd., Watford, UK). Pots were arranged outdoors in a randomised complete block design with six replicates per hybrid × treatment combination.

Pots were placed in perforated 1 m x 1 m square trays (Garland products ltd, Kingswinford, UK) to prevent waterlogging. For the first two months, irrigation was applied manually two to three times per week using an overhead sprinkler system. Thereafter, an automated capillary irrigation system delivered water three times daily from micro-irrigation pipes below and via matting placed inside the trays. Occasional overhead watering was applied during extended dry periods. Seasonal environmental conditions during the experiment are summarised in Supplementary Figure S6.

Measurements were taken at intervals from 4th June to 4th November 2024 (Year 1) and from 17th May to 17th June 2025 (Year 2). Stretched height (maximum canopy height) was measured from soil surface to the tallest leaf tip (Nunn *et al.*, 2017). Ligule height was also recorded as an indicator of structural stem elongation from soil to base of the last fully expended leaf (indicated by the presence of a ligule). Growth rate (GR; cm day⁻¹) was calculated as the slope of the growth curves. Tiller number, leaf number, survival (Year 2), and emergence timing were recorded.

At the end of each growing season, aboveground biomass (AGB) was harvested at approximately 5 cm above soil level. In Year 2, belowground biomass (rhizomes and roots) were excavated, washed, and dried. All biomass samples were oven-dried at 90 °C

to constant weight. Moisture content was calculated as (fresh weight – dry weight) / fresh weight × 100.

$$MC = \frac{FW - DW}{FW} \cdot 100$$

Field experiment

The field experiment was conducted at Ness Botanic Gardens (53°16'19.56"N, 3°2'44.16"W). Climatic conditions during the growing season are summarised in Supplementary Figure S6.

The field experiment followed a randomised complete block design including hybrid (three levels), PGR/BS treatment (five levels), and block (n = 4). Each hybrid × treatment combination was represented by three plants per block (see supplementary Figure S7 for field plan). Plants were spaced at 0.3 m within plots (equivalent to approximately 11 plants m⁻²), with 0.5 m between plots and a 0.3 m perimeter buffer planted with *Miscanthus* to minimise edge effects. Newly grown treated plugs were cut down to 3 cm and transplanted on 7th May 2025, and no additional PGR/BS was applied after transplanting. On that day, the plot was well irrigated with water, and irrigation was applied once a week for approximately two months after transplanting.

Using the same protocols as in the pot experiment, stretched height, ligule height, growth rate, and tiller number were measured. In addition, percentage regrowth (to account for plugs that re-emerged after cutting and transplanting) and survival (to account for plugs that re-emerged and fully established) were recorded. Aboveground biomass was assessed at harvest. Owing to the large size of field-grown plants, total fresh biomass per plot was first weighed using a portable handheld digital balance (FREETO, Shenzhen, China). A representative subsample (typically 200–500 g fresh weight per plot, approximately 10–20% of total biomass) was collected, ensuring proportional inclusion of stem and leaf material, weighed fresh, oven-dried at 90 °C to constant mass, and used to estimate total dry biomass and moisture content for each plot based on fresh-to-dry weight ratios.

Physiological and compositional analyses

Ash content was determined by combusting oven-dried stem material at 540 °C for 4 hours in a furnace (Carbolite Gero Ltd., Hope, UK). Samples were weighed before and after combustion, and ash percentage was expressed on a dry-weight basis. The resulting ash residue was subsequently analysed using X-ray fluorescence (XRF; Epsilon 1, Malvern Panalytical Ltd., Malvern, UK) to quantify elemental composition. Major ash-forming elements (K, Ca, Mg, Si, P, S, Cl, and Na) and minor/potentially toxic elements (Al, As, Fe, Pb, Mn, Ni, Cr, Cu, Co, V, Zn, and Ti) were all quantified and summed as these two groups following the approach described by Endriss *et al.* (2024).

Higher heating value (HHV) was determined using a Parr 6050 compensated jacket calorimeter (Parr, instrument company, Illinois, USA). Chopped dry samples (approximately 1 g) were combusted under oxygen pressure following manufacturer protocols. The heat of combustion (HoC) was determined and expressed as HHV (MJ kg⁻¹) of dry weight following ASTM D5865 and ISO 18125 standard procedures. Also, approximately 100 mg of oven-dried, ground stem material was analysed for total carbon and nitrogen using a Skalar PRIMACS™ SNC-100 analyser (Breda, Netherlands) following manufacturer instructions. Carbon-to-nitrogen ratio was calculated on a mass basis.

Statistical analysis

All statistical analyses were conducted in R (version 4.6.0). Data were assessed for normality and homogeneity of variance using residual diagnostics and formal tests. Repeated growth measurements were analysed using linear mixed-effects models fitted with the *lmer* function from the *lme4* package (Bates *et al.*, 2015). Models included PGR/BS treatment, hybrid, time (measurement date or growth stage), and their interactions as fixed effects, with block included as a random effect.

Aboveground biomass and final endpoint height from the pot and field experiments were analysed jointly across environments using linear mixed-effects models, with environment, PGR/BS treatment, hybrid, and their interactions as fixed effects. Block was treated as a random effect nested within environment to account for the separate blocking structures used in the pot and field experiments. Biomass was log-transformed prior to analysis to improve compliance with model assumptions. Estimated marginal

means were used for post-hoc comparisons, with Tukey adjustment for pairwise comparisons and Dunnett adjustment for comparisons of each PGR/BS treatment against the control.

Growth trajectories were partitioned into developmental phases based on days after planting (DAP), consistent with phase transitions observed in this experiment: early establishment (≤ 30 DAP), characterised by emergence and initial canopy formation; rapid elongation (31–80 DAP), corresponding to the period of steep stem growth; and saturation/late phase (> 80 DAP), during which growth rates declined and canopy expansion approached a plateau. Growth rate (GR) during the rapid phase was derived from growth curves and analysed using factorial ANOVA within each environment. Germination timing was analysed using Kruskal–Wallis tests where parametric assumptions were not met.

Single-time-point variables, including biomass components, ash content, MAE and M-PTE sums, HHV, carbon concentration, nitrogen concentration, and C:N ratio, were analysed using factorial ANOVA with hybrid and PGR/BS treatment as fixed effects and block as a random effect. Where significant effects were detected, means were separated using Tukey's HSD at $\alpha = 0.05$.

All tabulated data are presented as mean $\pm$ standard error unless otherwise stated.

RESULTS

Structural growth dynamics across environments

Across hybrids and environments, stretched height followed a classical sigmoidal trajectory comprising an early establishment phase, a steep rapid elongation phase, and a saturation phase (Figure 1; Figure S1; Figure S2). Field-grown plants exhibited a sharper rapid phase and attained greater absolute heights than pot-grown plants (Figure 1a–b), consistent with higher growth rates (GR) under field conditions (Table 2). Despite this shared developmental pattern, treatment ranking and the degree of separation were strongly hybrid and environment-dependent, with treatment effects expressed predominantly during rapid elongation and consolidated at saturation. ANOVA showed that treatment effects were not significant during the early growth phase in either the Year

1 pot or Year 1 field experiment ($p > 0.05$) but became significant during the rapid and late growth phases in the Year 1 pot experiment and during the late growth phase in the Year 1 field experiment (Table S1).

In Year 1 pots (Figure 1a), treatment separation became apparent at the onset of rapid growth. In GNT14, Micropull (MP) led from rapid phase through to late growth phase, followed by Liquid Ice (LI), with Control tracking slightly below LI. IAA and NAA lagged during rapid elongation; although IAA began lower than NAA, it overtook NAA during late growth. These trajectory patterns were concordant with GR values (Table 2), and ANOVA analysis of elongation growth from the rapid growth phase showed significant treatment and hybrid effects ($p < 0.05$) in Pot Year 1 (Table S1). In GNT3, LI separated clearly from the clustered treatments and maintained dominance into late phase; IAA showed transient suppression during rapid growth but converged during late growth. In GNT43, LI, MP, and NAA separated early, but LI tailed off during late growth, resulting in lower variability of Control and IAA with MP and NAA by saturation. Thus, in pots, late-phase variability was most evident in GNT14 and GNT3, whereas GNT43 exhibited lower variability during the late growth phase.

In Year 1 field trajectories (Figure 1b), treatment ranking shifted. ANOVA indicated significant late-phase treatment $\times$ Hybrid interactions in the field ($p < 0.05$), whereas early-phase effects were not significant ($p > 0.05$; Table S1). In GNT14, treatments remained tightly packed through early and rapid phases, with modest separation emerging primarily at saturation. In contrast, GNT3 exhibited the clearest field separation: IAA separated during rapid elongation and remained the tallest at late phase, followed by LI, MP, NAA, and Control. This ordering corresponded to GR values (Table 2). In GNT43, field trajectories showed substantial overlap with MP maintaining a lower growth trajectory during the late growth phase than the other treatments.

Collectively, these data (pot and field) demonstrate that PGR/BS-mediated modulation of canopy development is expressed chiefly through rapid growth phase elongation, but the magnitude and direction of treatment effects depend on hybrid identity (genotype) and environmental context.

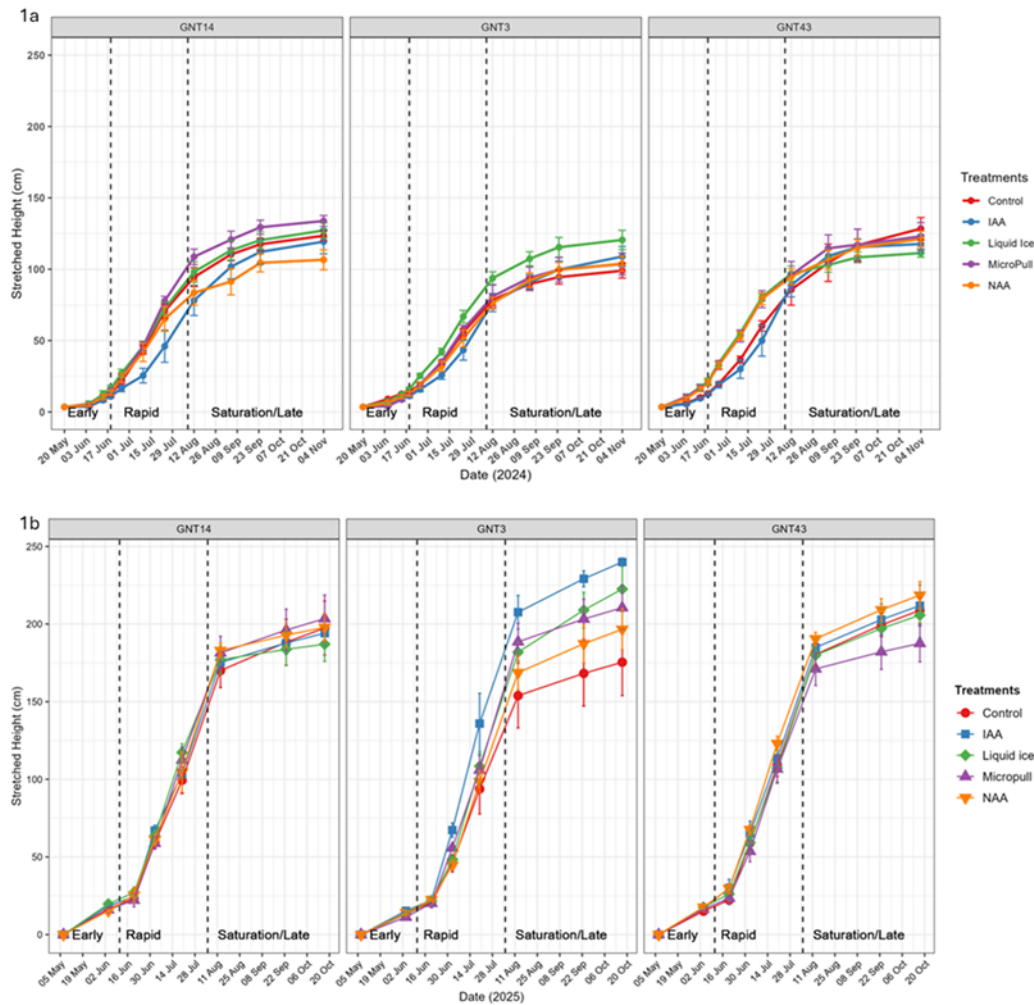


Figure 1. Seasonal stretched height trajectories across environments. Stretched height (cm) of three *Miscanthus* hybrids (GNT14, GNT3, GNT43) under five plant growth regulator (PGR) treatments in (a) pots (Year 1, 2024) and (b) field conditions (Year 1, 2025). Dashed vertical lines indicate developmental phases (early, rapid elongation, saturation/late). Points represent means $\pm$ SE (pots: $n = 6$; field: $n = 4$ blocks). Treatments: Control, IAA, Liquid Ice (LI), MicroPull (MP), NAA.

Days to emergence (Figure S3) and tiller number (Figure S5) provided mechanistic context for these structural patterns. ANOVA indicated no significant effect of treatments on days to emergence in either environment ($p > 0.05$; Table S1). Although treatments differed in emergence timing, these differences were not consistently maintained during subsequent canopy development, with treatment differences becoming more pronounced during the rapid elongation phase. Tiller number exhibited significant treatment effects ($p < 0.05$) in pot Year 1 but not in field conditions ($p > 0.05$; Table S1), and did not uniformly covary with stretched height, supporting the interpretation that elongation kinetics rather than tillering intensity underpinned most structural differences.

Biomass accumulation and allocation

First-year aboveground biomass responses

In the Year 1 pot experiment, aboveground biomass (AGB) broadly reflected the structural separation observed in stretched-height trajectories (Figure 2a). In GNT14, MP produced significantly greater AGB than the Control ($P < 0.05$), whereas LI did not differ significantly from the Control despite strong height performance; IAA and NAA produced numerically lower AGB than the Control. In GNT3, LI produced significantly greater AGB than all other treatments, while the remaining treatments did not differ significantly from the Control. In GNT43, MP similar to GNT14 produced the highest AGB. These patterns indicate that under controlled conditions, rapid-phase structural advantages can translate into biomass gains, although the dominant treatment differs among hybrids and height advantages do not invariably translate into AGB gains (notably in GNT14 where LI improved height yet did not clearly outperform Control for AGB).

In Year 1 field biomass, treatment separations were not significant ($p > 0.05$; Figure 2b). Although numerical ranking was apparent in some hybrids (e.g., in GNT14, Control tended to exceed other treatments; in GNT43, MP was numerically low), differences were not statistically significant. Given the high germination and survival observed across treatments (Figure S4), the limited separation between treatments at the first-season field harvest was not associated with substantial treatment-dependent plant loss. Treatment differences observed in the pot experiment were less pronounced under field conditions, indicating that some treatment responses were not consistently maintained following field establishment. The contrast between treatment separation in field height, particularly in GNT3 (Figure 1b), and the comparatively weak treatment separation in AGB (Figure 2b) indicates that treatment effects on structural stature were not consistently reflected in biomass accumulation under field conditions.

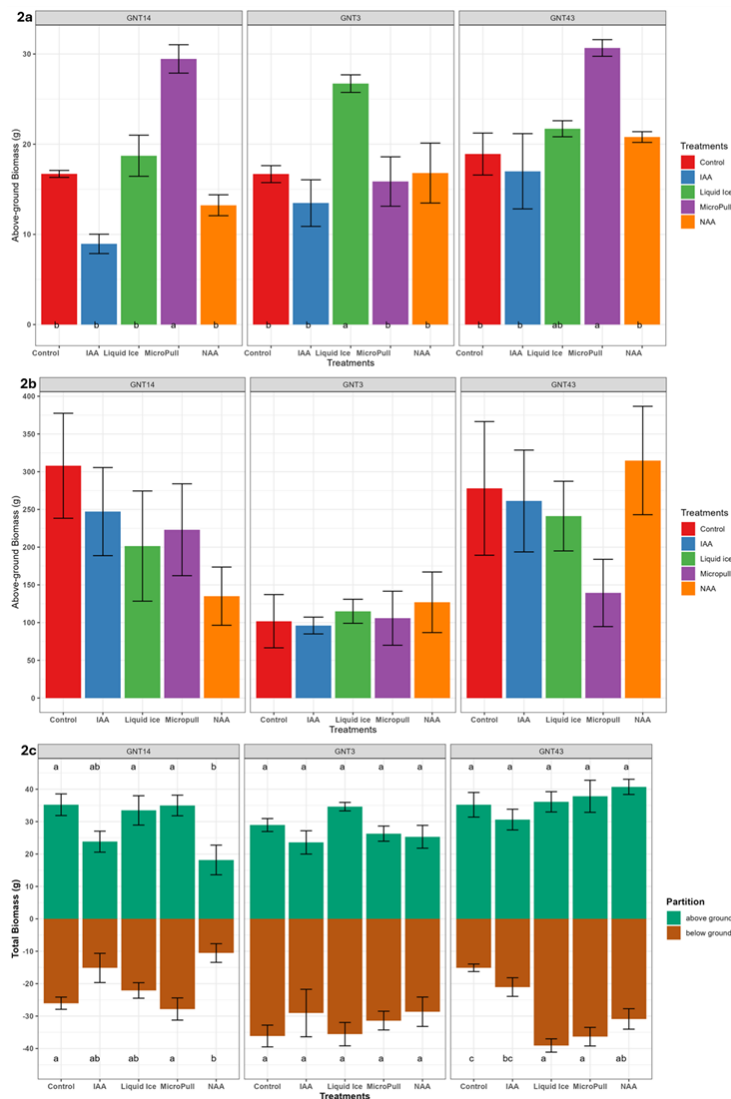


Figure 2. Biomass accumulation and allocation across environments and seasons. (a) Year 1 pot above-ground biomass (AGB). (b) Year 1 field AGB. (c) Year 2 pot above-ground and below-ground biomass (BGB). Bars represent means $\pm$ SE (field; $n = 4$, pot; $n = 6$). Letters indicate significant differences among treatments within hybrid and environment (Tukey's HSD, $\alpha = 0.05$). In panel (c), below-ground biomass is plotted below zero to visualise allocation patterns. Treatments as in Figure 1.

Second-year biomass persistence and belowground allocation

Second-year pot harvests revealed hybrid-specific patterns of persistence (Figure 2c). In GNT14, treatment effects contrasted with the Year 1 pattern: NAA produced the lowest total biomass (29 g), while MP, LI, and Control maintained comparatively high values (57 to 70 g), and IAA remained intermediate (39 g). This pattern was consistent across both above- and below-ground partition, indicating a negative carryover effect of NAA and sustained performance under MP.

In GNT3, neither aboveground nor belowground biomass showed statistically significant difference ($p > 0.05$) among treatments in Year 2 (Figure 2c), consistent with attenuation

of earlier treatment separation. In GNT43, Year 2 aboveground biomass remained largely overlapping among treatments, whereas belowground biomass showed significant separation ($p < 0.05$; Table S1), with Control reduced relative to most treatments. Together, the second-year results indicate that treatment responses were not consistently maintained from the first-year pot experiment into the second year and varied among hybrids and biomass components, with some treatment differences more evident in belowground than aboveground biomass.

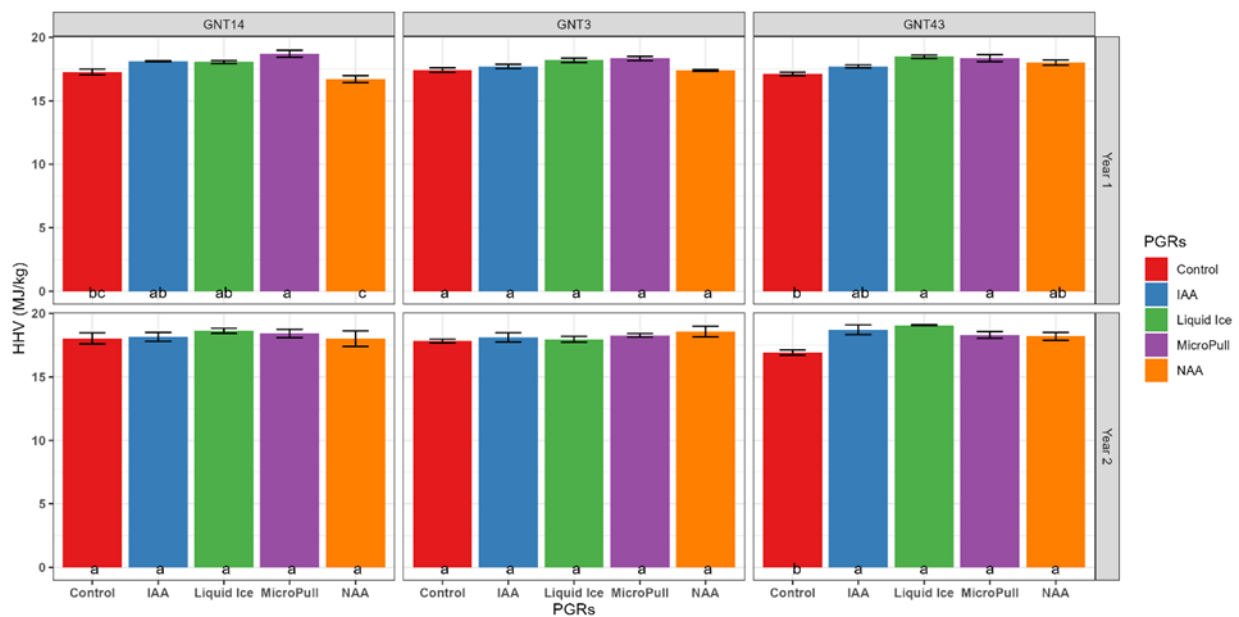


Figure 3. Higher heating value (HHV) of stem biomass. Stem HHV (MJ kg^{-1} dry weight) of three *Miscanthus* hybrids under five PGR treatments. Upper panels show Year 1 pot data; lower panels show Year 2 pot data. Bars represent means $\pm$ SE ($n = 4$). Letters denote significant treatment differences within hybrid and year (Tukey's HSD, $\alpha = 0.05$).

Biomass quality traits

In contrast to growth and biomass traits, biomass quality parameters exhibited limited and inconsistent treatment sensitivity (Figures 3–5; Table 2). Higher heating value (HHV) differences were statistically significant ($p < 0.05$) in select hybrid–year groupings but small in magnitude relative to differences observed in biomass. In GNT14 (Year 1 pots), MP was among the upper group and NAA was lowest; these differences diminished in Year 2. GNT3 showed no statistical significant difference ($p > 0.05$) across years. In GNT43, LI and MP exceeded Control in Year 1, while in Year 2 all treatments were significantly higher ($p < 0.05$) than the Control (Figure 3).

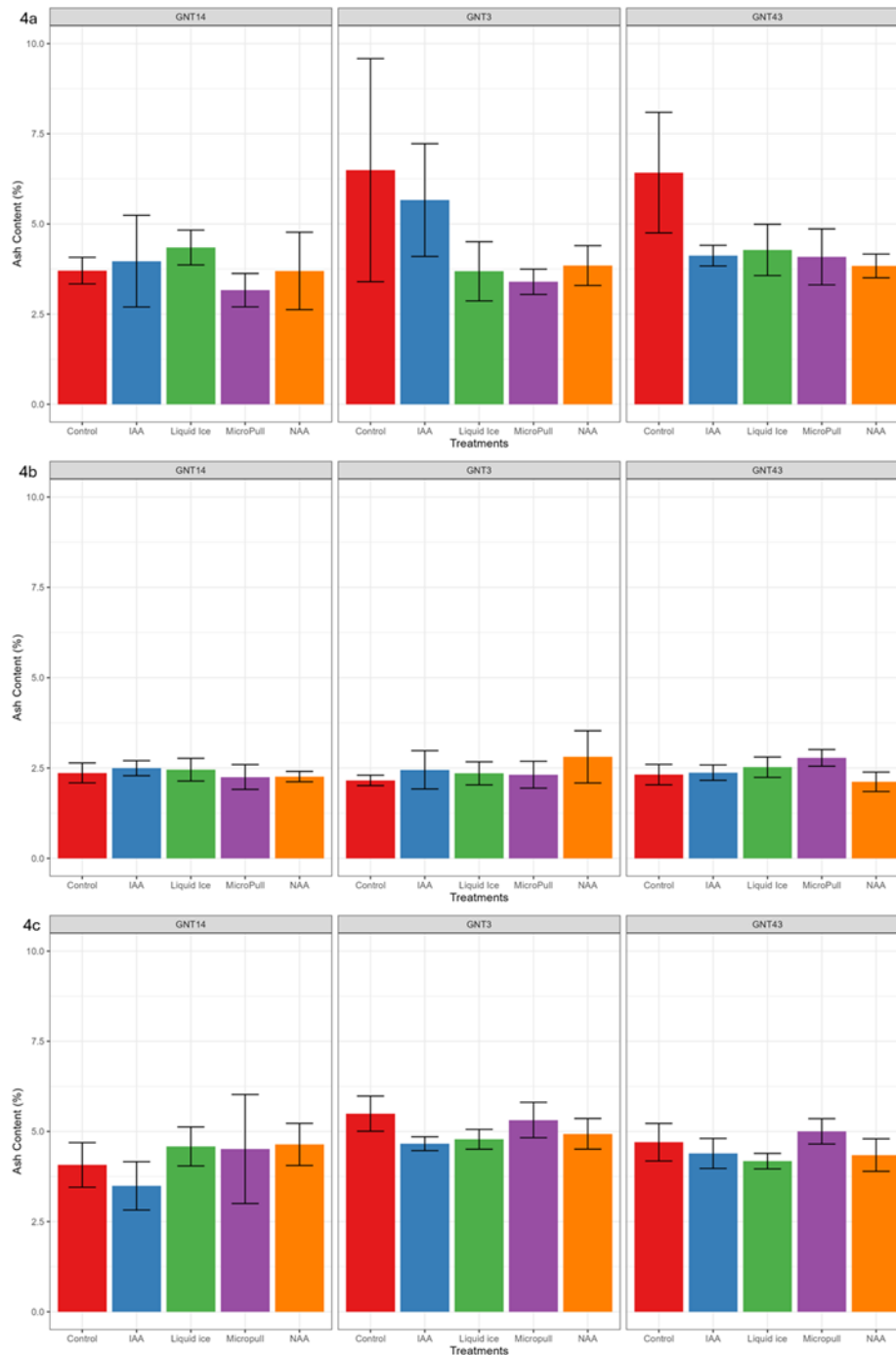


Figure 4. Stem ash content across environments. Ash content (%) of stem biomass in (a) Year 1 pots, (b) Year 2 pots, and (c) field conditions. Ash was determined after combustion at 540 °C and expressed on a dry-weight basis. Bars represent means $\pm$ SE ($n = 4$).

Ash content showed no significant treatment effects ($p > 0.05$; Table S1) across pot or field experiment (Figure 4), with a pronounced year effect: ash content was lower and more homogeneous in Year 2 (pots) relative to Year 1 (pot and field). Carbon concentration was stable across treatments and environments (Table 2), while nitrogen concentration decreased in Year 2, yielding higher C:N ratios (Figure 5). Despite these

temporal shifts, C:N did not show statistically significant treatment separation ($p > 0.05$) across both environments (Table S1). Overall, these results indicate that the treatments altered structural growth and biomass more strongly than compositional quality traits, and quality parameters were driven predominantly by hybrid identity and season.

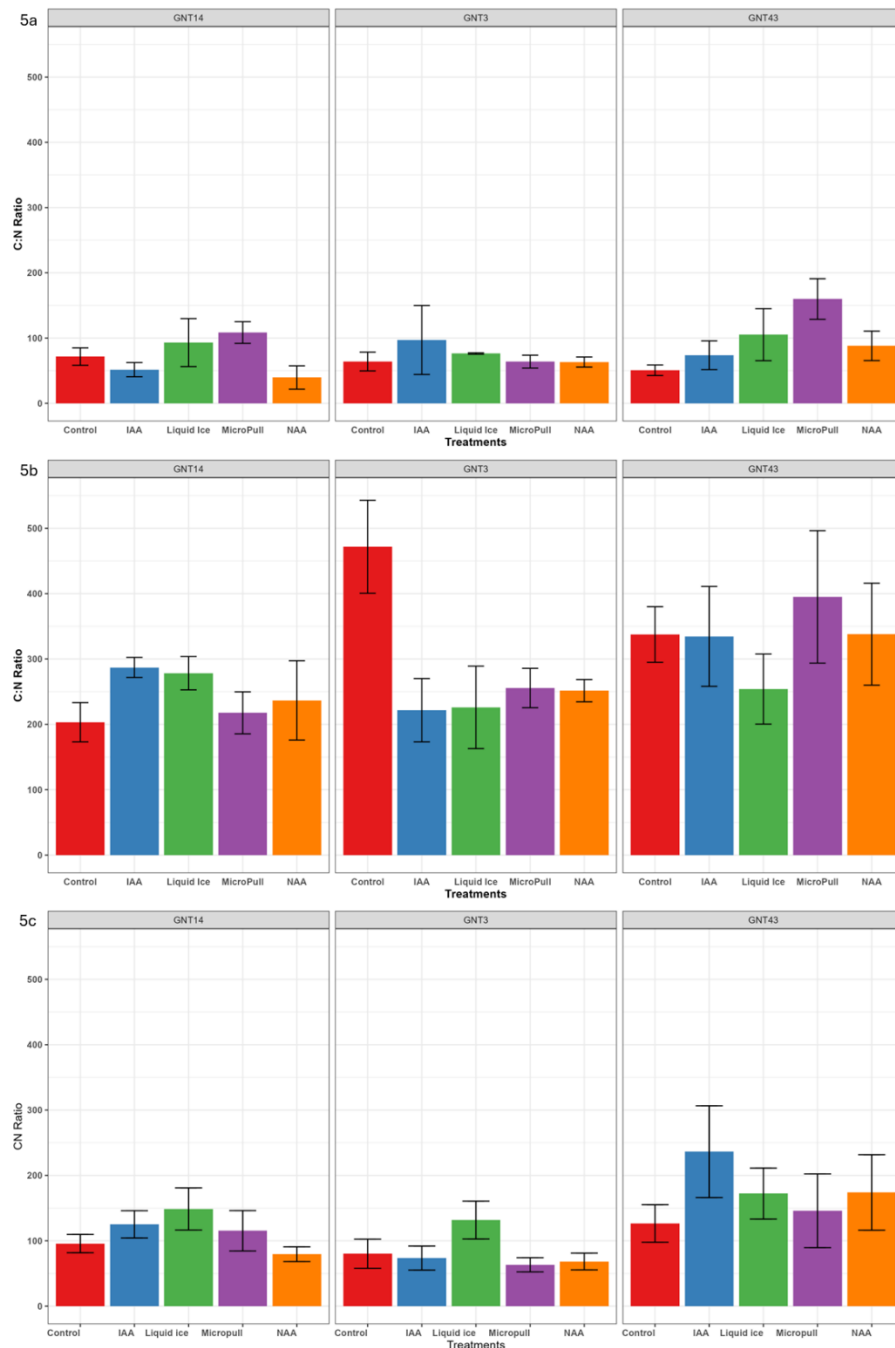


Figure 5. Stem carbon-to-nitrogen (C:N) ratio. C:N ratio (mass basis) of stem biomass in (a) Year 1 pots, (b) Year 2 pots, and (c) field conditions. Values represent means $\pm$ SE ($n = 4$). C and N concentrations were determined using elemental analysis.

Cross-environment growth and biomass responses

Endpoint height differed strongly between the pot and field experiments ($F_{1,8 \cdot 05} = 192.66$, $P < 0.001$), whereas the overall effects of PGR/BS treatment ($F_{4,105 \cdot 27} = 1.20$, $P = 0.315$) and hybrid ($F_{2,105 \cdot 26} = 1.10$, $P = 0.337$) were not significant. However, significant environment $\times$ hybrid ($F_{2,105 \cdot 26} = 7.05$, $P = 0.0013$) and PGR/BS $\times$ hybrid interactions ($F_{8,105 \cdot 26} = 3.78$, $P < 0.001$) indicated that endpoint height responses varied among hybrids. Neither the environment $\times$ PGR/BS interaction ($F_{4,105 \cdot 27} = 1.82$, $P = 0.130$) nor the environment $\times$ PGR/BS $\times$ hybrid interaction ($F_{8,105 \cdot 26} = 1.57$, $P = 0.143$) was significant.

Within the pot environment, none of the PGR/BS treatments differed significantly from the control for any hybrid following Dunnett adjustment. In the field environment, however, GNT3 showed significantly greater endpoint height following IAA (+64.5 cm, $P < 0.001$), Liquid Ice (+47.1 cm, $P = 0.002$) and MicroPull (+37.9 cm, $P = 0.028$) application relative to the control, whereas NAA did not differ from the control ($P = 0.297$). No significant treatment-control differences were detected for GNT14 or GNT43 in the field. Endpoint height was also greater in the field than in the pot experiment across all hybrid $\times$ treatment combinations, with environment contrasts ranging from 59.9 to 131.7 cm and all comparisons significant at $P < 0.001$ (Figure 6).

Biomass showed a similarly strong difference between experiments but a more complex pattern of treatment and hybrid responses. Biomass differed significantly between environments ($F_{1,7 \cdot 99} = 685.06$, $P < 0.001$) and among hybrids ($F_{2,112} = 11.38$, $P < 0.001$), whereas the overall effect of PGR/BS treatment was not significant ($F_{4,112} = 1.11$, $P = 0.355$). Significant environment $\times$ PGR/BS ($F_{4,112} = 4.61$, $P = 0.0017$), environment $\times$ hybrid ($F_{2,112} = 8.14$, $P < 0.001$), and PGR/BS $\times$ hybrid interactions ($F_{8,112} = 2.09$, $P = 0.042$) indicated that biomass responses varied according to both growing environment and hybrid. The three-way environment $\times$ PGR/BS $\times$ hybrid interaction was not significant ($F_{8,112} = 1.25$, $P = 0.279$).

Despite these interaction effects, none of the individual PGR/BS treatments differed significantly from the control within any hybrid $\times$ environment combination after Dunnett adjustment. The closest treatment-control comparisons were observed for MicroPull in pot-grown GNT14 ($P = 0.061$), MicroPull in pot-grown GNT43 ($P = 0.062$), and NAA in field-

grown GNT14 ($P = 0.050$), but none exceeded the significance threshold. Biomass was significantly greater in the field than in the pot experiment for every hybrid $\times$ treatment combination, with all environment contrasts significant at $P < 0.001$ (Figure 6).

Together, these results demonstrate a strong difference between the pot and field experiments in both endpoint height and biomass, while treatment responses were dependent on hybrid and, for biomass, growing environment. The strongest treatment-related height responses occurred in field-grown GNT3, where IAA, Liquid Ice and MicroPull increased endpoint height relative to the control, but these differences were not accompanied by significant increases in biomass. Thus, greater treatment-associated structural growth did not consistently translate into greater biomass production across hybrids or growing environments.

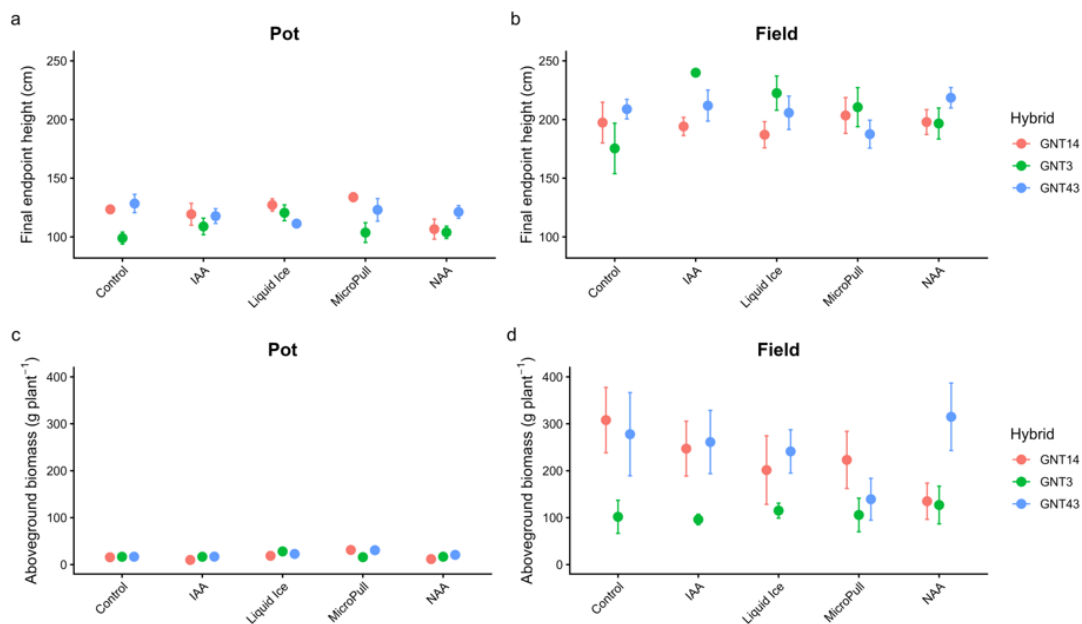


Figure 6. Cross-environment responses of *Miscanthus* hybrids to PGR/BS treatments. Final endpoint height (a, b) and aboveground biomass (c, d) of GNT14, GNT3 and GNT43 in pot (a, c) and field (b, d) experiments following application of Control, IAA, Liquid Ice, MicroPull and NAA. Points represent means $\pm$ SE (field: $n = 4$, pot $n = 6$). Different colours indicate *Miscanthus* hybrids.

Table 2. Showing effect of pgr treatment on establishment and biomass quality of three *Miscanthus* hybrids (GNT3, GNT14, GNT43). GR: growth rate, M-PTE: minor and potentially toxic elements, MAE: major ash-forming elements.

Environment	Year	Hybrid	PGR Treatment	GR (cm/day)	* M-PTE (ppm)	* MAE (ppm)	C	N
Pots	1	GNT14	Control	0.86±0.01 ^b	10.68±0.38 ^{bc}	98.82±0.04 ^{bc}	44.00±0.15 ^a	0.72±0.19 ^a
			IAA	0.70±0.03 ^d	14.77±1.47 ^a	98.42±0.15 ^d	46.20±1.18 ^a	0.74±0.29 ^a
			Liquid ice	0.90±0.01 ^{ab}	06.75±0.12 ^d	99.24±0.00 ^a	45.00±0.73 ^a	0.74±0.26 ^a
			Micropull	0.94±0.01 ^a	11.57±0.22 ^b	98.74±0.02 ^c	44.70±0.47 ^a	0.44±0.06 ^a
			NAA	0.74±0.03 ^c	08.60±0.02 ^{cd}	99.06±0.00 ^{ab}	44.30±0.97 ^a	2.06±0.86 ^a
		GNT3	Control	0.64±0.01 ^c	07.82±0.50 ^b	99.12±0.05 ^a	44.10±0.26 ^a	0.77±0.12 ^a
			IAA	0.66±0.01 ^{bc}	15.26±1.74 ^a	98.36±0.18 ^b	42.70±2.44 ^a	0.60±0.30 ^a
			Liquid ice	0.84±0.02 ^a	13.97±0.43 ^a	98.51±0.42 ^b	45.60±0.90 ^a	0.51±0.09 ^a
			Micropull	0.71±0.01 ^b	14.41±0.43 ^a	98.45±0.35 ^b	46.00±0.95 ^a	0.79±0.15 ^a
			NAA	0.67±0.01 ^{bc}	07.87±0.05 ^b	99.12±0.00 ^a	44.90±0.57 ^a	0.74±0.07 ^a
		GNT43	Control	0.81±0.02 ^{bc}	10.83±0.03 ^a	98.81±0.00 ^b	44.80±0.89 ^a	0.70±0.30 ^a
			IAA	0.76±0.02 ^c	08.31±0.22 ^{ab}	99.05±0.03 ^{ab}	44.30±0.35 ^a	0.79±0.22 ^a
			Liquid ice	0.86±0.01 ^{ab}	07.83±0.14 ^b	99.12±0.02 ^a	43.80±0.17 ^a	0.65±0.23 ^a
			Micropull	0.89±0.02 ^a	09.79±0.05 ^{ab}	99.92±0.00 ^{ab}	44.20±0.77 ^a	0.31±0.06 ^a
			NAA	0.90±0.01 ^a	10.66±0.07 ^a	98.84±0.00 ^b	44.40±1.07 ^a	0.71±0.29 ^a
	2	GNT14	Control	0.98±0.02 ^a	05.59±0.59 ^a	99.31±0.06 ^a	45.40±0.20 ^a	0.18±0.07 ^a
			IAA	0.97±0.04 ^a	06.02±0.94 ^a	99.29±0.10 ^a	45.70±0.54 ^a	0.08±0.05 ^a
			Liquid ice	0.97±0.04 ^a	05.48±0.15 ^a	99.34±0.02 ^a	45.40±0.19 ^a	0.10±0.06 ^a
			Micropull	1.01±0.01 ^a	06.64±0.98 ^a	99.21±0.12 ^a	45.40±0.35 ^a	0.13±0.05 ^a
			NAA	0.95±0.05 ^a	05.10±0.07 ^a	99.40±0.00 ^a	45.10±0.43 ^a	0.22±0.05 ^a
		GNT3	Control	0.72±0.03 ^b	11.10±0.38 ^a	98.77±0.04 ^c	45.50±0.12 ^a	0.08±0.02 ^a
			IAA	0.78±0.05 ^b	08.88±0.26 ^b	98.99±0.02 ^b	45.20±0.55 ^a	0.24±0.05 ^a
			Liquid ice	0.94±0.06 ^a	08.16±0.21 ^b	99.07±0.02 ^b	45.10±0.50 ^a	0.10±0.07 ^a
			Micropull	0.81±0.03 ^{ab}	05.95±0.10 ^c	99.28±0.00 ^a	45.50±0.39 ^a	0.08±0.04 ^a
			NAA	0.76±0.05 ^b	08.38±0.12 ^b	99.06±0.01 ^b	45.50±0.31 ^a	0.11±0.06 ^a
		GNT43	Control	1.11±0.02 ^a	04.60±0.23 ^c	99.37±0.03 ^a	45.70±0.24 ^a	0.10±0.04 ^a
			IAA	0.99±0.04 ^{ab}	05.29±0.08 ^{bc}	99.33±0.00 ^a	45.10±0.22 ^a	0.17±0.05 ^a
			Liquid ice	0.96±0.03 ^b	06.86±0.99 ^{ab}	99.20±0.10 ^{ab}	45.10±0.32 ^a	0.23±0.09 ^a
			Micropull	1.03±0.03 ^{ab}	08.50±0.31 ^a	99.02±0.03 ^b	48.20±2.64 ^a	0.11±0.06 ^a
			NAA	1.01±0.02 ^{ab}	05.97±0.12 ^{bc}	99.25±0.01 ^a	45.40±0.34 ^a	0.17±0.05 ^a
Field	1	GNT14	Control	1.28±0.04 ^c	26.20±4.81 ^a	14.25±2.23 ^a	48.10±1.46 ^a	0.55±0.10 ^a
			IAA	1.24±0.02 ^c	23.80±4.83 ^a	14.25±2.27 ^a	46.20±0.44 ^a	0.40±0.06 ^a
			Liquid ice	1.33±0.03 ^{bc}	26.10±4.99 ^a	14.25±2.27 ^a	47.40±0.45 ^a	0.38±0.10 ^a
			Micropull	1.43±0.04 ^a	27.60±5.32 ^a	14.25±2.25 ^a	46.80±0.73 ^a	0.49±0.12 ^a
			NAA	1.40±0.03 ^{ab}	26.00±5.19 ^a	14.25±2.28 ^a	46.30±0.20 ^a	0.42±0.20 ^a
		GNT3	Control	1.20±0.04 ^a	23.00±4.51 ^a	14.25±2.25 ^a	45.30±0.88 ^a	0.71±0.19 ^a
			IAA	1.73±0.02 ^a	27.60±5.83 ^a	14.25±2.23 ^a	46.30±0.10 ^a	0.50±0.23 ^a
			Liquid ice	1.51±0.06 ^b	25.90±5.53 ^a	14.25±2.25 ^a	47.20±0.58 ^a	0.43±0.12 ^a
			Micropull	1.35±0.06 ^c	27.90±5.83 ^a	14.25±2.24 ^a	45.40±1.04 ^a	0.78±0.13 ^a
			NAA	1.22±0.04 ^d	25.50±4.94 ^a	14.25±2.23 ^a	46.60±0.20 ^a	0.78±0.18 ^a
		GNT43	Control	1.56±0.02 ^{ab}	24.90±5.43 ^a	14.25±2.27 ^a	46.40±0.55 ^a	0.44±0.11 ^a
			IAA	1.44±0.03 ^b	25.60±5.07 ^a	14.25±2.26 ^a	47.70±0.39 ^a	0.25±0.05 ^a
			Liquid ice	1.47±0.03 ^b	23.80±5.31 ^a	14.25±2.26 ^a	49.30±2.36 ^a	0.39±0.02 ^a
			Micropull	1.30±0.02 ^c	24.40±5.23 ^a	14.25±2.25 ^a	47.20±0.20 ^a	0.51±0.19 ^a
			NAA	1.64±0.01 ^a	25.40±5.49 ^a	14.25±2.24 ^a	47.00±0.25 ^a	0.35±0.09 ^a

* All values are divided by 10 except field values. Letters indicate significant differences among treatments within each hybrid, as determined by Tukey's HSD test ($p < 0.05$). Treatments sharing the same letter within a hybrid, year and environment are not statistically different, $n = 6$ & 4 for pot & field environment, respectively.

DISCUSSION

Treatments modulated elongation kinetics rather than establishment timing

The treatments did not alter when plants emerged or established, but they strongly influenced how fast they elongated once rapid growth began. Differences in emergence timing were small and unrelated to final canopy structure. Instead, treatment effects became visible at the start of rapid elongation and continued to widen through late growth phase. This pattern is consistent with the sigmoidal growth trajectory observed in plants, including *Miscanthus*, where variation during the rapid elongation phase can strongly influence subsequent canopy development and biomass accumulation (Poorter *et al.*, 2012, Robson *et al.*, 2025). The growth-phase analysis reinforces this interpretation

by showing that treatment separation consistently aligned with the rapid elongation window rather than early establishment. Robson et al. (2025) similarly reported that, among diverse *Miscanthus* genotypes, earlier seasonal growth was associated with greater dry biomass yield when the duration of logarithmic growth was similar, although relationships between stem-growth traits and accumulated dry biomass were moderate overall. The present results extend this observation by showing that exogenous PGR and BS treatments primarily altered the rapid elongation phase, while treatment effects on final biomass varied among hybrids.

Field-grown plants exhibited a steeper rapid phase and greater final height than pot-grown plants, consistent with the greater rooting depth and resource access known to enhance *Miscanthus* growth in field environments (Clifton-Brown *et al.*, 2001). The sharper field trajectories likely reflect reduced rooting restriction and greater resource availability under field conditions relative to pots. The observation that treatment divergence was most pronounced during rapid elongation supports a mechanistic interpretation in which treatments altered the gain of elongation response systems rather than simply shifting developmental onset (Santner & Estelle, 2009; Chapman & Estelle, 2009).

Hybrid-specific differences in treatment responses indicate that responsiveness to exogenous regulators is influenced by genotype and physiological context. Auxin-mediated growth responses are known to depend on the cellular and developmental context in which auxin is perceived and transduced, with differences in signalling networks contributing to variation in response (Santner & Estelle, 2009; Chapman & Estelle, 2009). Similar genotype-dependent variation in physiological responses has been reported in *Miscanthus* under environmental stress (Clifton-Brown *et al.*, 2004). Thus, the contrasting responses of GNT3, GNT14 and GNT43 are more appropriately interpreted as evidence of genotype-dependent responsiveness rather than uniform treatment efficacy. The environment-sensitive nature of these genotype × treatment interactions further indicates that treatment responses were contingent on growing conditions.

Improved height does not universally translate into biomass gain

While first-year pot biomass broadly reflected structural ranking, the relationship was not deterministic. In GNT3, for example, IAA produced a greater final stretched height than the Control but did not result in greater aboveground biomass. This indicates that canopy elongation and biomass accumulation are related but not equivalent processes, with biomass accumulation additionally dependent on leaf area deployment, photosynthetic capacity and carbon partitioning (Körner, 2003; Poorter *et al.*, 2012). This interpretation is consistent with evidence from *Miscanthus* showing that early stem growth can contribute to final biomass accumulation, while interactions among stem traits can produce contrasting growth strategies and weaken the relationship between individual structural traits and biomass production (Robson *et al.*, 2012; 2019; 2025). In particular, similar yields may arise from contrasting combinations of stem number and stem length, such that greater height does not necessarily correspond to proportionally greater biomass (Robson *et al.*, 2019). The inconsistent correspondence between stretched height and biomass observed here therefore suggests that treatment-induced differences in structural development were not necessarily translated proportionally into biomass accumulation.

Field conditions further weakened the linkage between structure and biomass. Despite pronounced height separation in GNT3 under IAA in the field, biomass differences were not statistically significant. Such decoupling is consistent with functional-equilibrium models, in which plants adjust biomass allocation in response to environmental constraints, potentially dampening structural advantages (Poorter *et al.*, 2012; Freschet *et al.*, 2018). Differences between controlled and field environments may provide an additional explanation, as field conditions introduce greater temporal and spatial environmental variability and can alter the relative expression of different growth traits (Poorter *et al.*, 2016). In *Miscanthus*, the traits explaining biomass production can also become more complex under environmental stress, with leaf area and physiological traits contributing alongside stem length and stem number (Malinowska *et al.*, 2020). Thus, the greater height observed under IAA did not necessarily translate into a statistically detectable biomass advantage, potentially because field conditions

influenced the multiple structural and physiological components contributing to biomass accumulation.

Taken together, the results presented in this study indicate that early canopy advantage does not reliably predict biomass yield. Instead, PGR/BS-induced elongation modifies architectural trajectories, and translation into biomass gain requires alignment with carbon assimilation and partitioning dynamics (Clifton-Brown *et al.*, 2001; Poorter *et al.*, 2012).

Persistence reflects allocation economics rather than structural carryover

Second-year biomass responses reveal that treatment effects are not simple extensions of first-year structural patterns. In GNT14, NAA did not perform poorly in Year 1 pots, yet produced the lowest above- and below-ground biomass in Year 2, indicating negative carryover from the initial structural response. This pattern suggests that early hormonal modulation may have altered reserve formation or rhizome allocation in ways that constrained regrowth, consistent with evidence that *Miscanthus* persistence depends strongly on rhizome carbohydrate reserves (Clifton-Brown & Lewandowski, 2000; Strullu *et al.*, 2011). In contrast, MP retained relatively strong performance across partitions, implying more balanced carbon economics.

The contrasting Year 2 outcomes among hybrids indicate that treatment effects extend beyond first-year structure and influence seasonal allocation trajectories. Carbon partitioning into belowground reserves is central to perennial regrowth capacity (Clifton-Brown *et al.*, 2001; Lewandowski *et al.*, 2000). Where structural enhancement is supported by adequate reserve accumulation, persistence is maintained. Where early elongation is not matched by reserve reinforcement, subsequent performance may decline. Thus, Year 2 outcomes reflect an integrated carbon-allocation strategy rather than simple structural carryover.

Studies in perennial biomass crops have similarly shown that end-of-season reserve accumulation can be a stronger determinant of subsequent regrowth than contemporaneous aboveground productivity. In *Miscanthus* × *giganteus*, nitrogen and biomass remobilisation dynamics were closely linked to belowground reserve formation and subsequent seasonal growth (Strullu *et al.*, 2011). Likewise, studies in switchgrass

demonstrated that management practices affecting reserve accumulation influenced stand persistence and regrowth in subsequent seasons (Lemus *et al.*, 2008; Ma *et al.*, 2001). The delayed treatment effects observed here are therefore consistent with the view that first-year structural responses in perennial grasses may not accurately predict subsequent productivity when allocation strategies differ among genotypes.

Notably, in GNT43, treatment separation was more evident in belowground biomass than in shoot mass, suggesting that allocation effects may be more sensitive indicators of persistence than aboveground traits. However, greater belowground allocation should not necessarily be interpreted as universally advantageous during establishment. Investment in roots and rhizomes may support water and nutrient acquisition, storage and subsequent regrowth, but may also reduce the proportion of assimilates available for rapid aboveground canopy development. This trade-off may be particularly important during establishment, when slower canopy development can increase vulnerability to weed competition and delay canopy closure. Thus, the value of increased belowground allocation is likely to depend on environmental conditions and developmental stage. This reinforces the importance of evaluating multicompartment responses when assessing treatment efficacy in perennial systems.

Biomass quality is comparatively buffered against structural manipulation

Despite substantial variation in structural growth and biomass accumulation, biomass compositional traits were comparatively stable. Differences in higher heating value were modest and inconsistent, ash content exhibited strong year effects but minimal treatment sensitivity, and carbon concentration remained stable across environments. The substantial inter-year variation in ash content may reflect differences in environmental conditions and nutrient availability between years, including possible differences in the mineral nutrient contribution of the compost substrate. However, this explanation cannot be confirmed because substrate composition and plant mineral uptake were not directly compared between years. Nevertheless, stability of biomass composition has been previously reported in *Miscanthus*, where biochemical composition varied relatively little across contrasting environments, nitrogen regimes, and harvest timings despite substantial variation in growing conditions (Arundale *et al.*,

2015). Likewise, seasonal changes in *Miscanthus* composition have been shown to be comparatively small relative to changes in biomass production (Peng *et al.*, 2017).

Conversely, harvest timing has been shown to exert a strong influence on combustion-related quality traits, including ash concentration, mineral content, and moisture dynamics (Iqbal *et al.*, 2017), highlighting that biomass quality can be sensitive to developmental and environmental processes when these directly affect nutrient remobilisation and tissue maturation. The limited treatment sensitivity observed in the present study therefore suggests that PGR and BS applications primarily influenced morphogenetic and allocation processes rather than the biochemical pathways governing fuel quality characteristics. Also, the absence of systematic treatment effects on quality suggests that structural enhancement does not inherently compromise bioenergy characteristics, a favourable outcome for agronomic optimisation.

Cross-environment treatment responses are hybrid-dependent

The cross-environment analysis showed that treatment responses varied between the pot and field experiments and among hybrids. Endpoint height differed strongly between experiments, with significant environment $\times$ hybrid and PGR/BS $\times$ hybrid interactions, whereas the environment $\times$ PGR/BS interaction was not significant. Biomass also differed strongly between experiments, with significant environment $\times$ PGR/BS, environment $\times$ hybrid and PGR/BS $\times$ hybrid interactions. These findings indicate that the expression of treatment responses varied among hybrids and between experimental conditions, rather than demonstrating a consistent treatment effect across the two experiments.

Similar discrepancies between controlled and field environments have been demonstrated experimentally. Forero *et al.* (2019) found that plant–soil feedback responses measured under greenhouse conditions were generally not correlated with those measured in the field, highlighting the limited predictive value of simplified experimental systems for complex field outcomes. Such differences reflect the greater environmental heterogeneity and biotic complexity of field conditions, which can alter both the magnitude and direction of biological responses. In the present study, the stronger treatment-related height responses observed in field-grown GNT3 were not accompanied by corresponding increases in biomass, further demonstrating that

treatment effects on structural growth did not necessarily translate into greater biomass production under field conditions.

Implications for screening and optimisation

Together, these results support the elongation and persistence hypotheses proposed in the Introduction, while demonstrating that treatment responses are strongly dependent on hybrid and growing environment. PGR and BS treatments primarily modified growth trajectories through changes in rapid growth phase elongation, with subsequent effects on biomass and allocation varying among hybrids and experimental conditions. Structural differences were particularly pronounced in the field for GNT3, whereas these differences did not consistently translate into greater biomass. This difference has direct consequences for screening strategies.

Most importantly, pot performance did not consistently predict the magnitude of treatment responses observed in the field. The strong environmental differences in endpoint height and biomass, together with significant environment $\times$ hybrid and environment $\times$ PGR/BS interactions for biomass, underscore the need for field validation alongside controlled screening. Integrating dynamic growth-phase analysis with direct cross-environment comparisons therefore provides a practical framework for identifying treatment responses that warrant further evaluation under field conditions.

In perennial bioenergy systems, optimisation must therefore consider three components simultaneously: (1) elongation kinetics, (2) seasonal carbon allocation, and (3) performance across contrasting growing environments. Treatments that enhance early elongation without compromising reserve formation, and that maintain beneficial responses under field conditions, represent the most promising candidates for scalable deployment.

CONCLUSION

This study demonstrates that PGR and BS responses in *Miscanthus* are governed by hybrid-specific modulation of rapid growth phase, downstream carbon allocation, and environmental condition. Structural separation was most strongly expressed during rapid growth, yet canopy dominance did not consistently translate into biomass gain or multi-

season persistence, underscoring the central role of allocation economics in perennial systems. Second-year responses revealed that early hormonal modulation can reshape reserve dynamics in hybrid-dependent ways, reinforcing that persistence reflects integrated carbon partitioning rather than simple structural carryover.

Critically, cross-environment comparisons showed that treatment responses were not uniform between pot and field experiments. Significant environment- and hybrid-dependent effects demonstrated that responses observed under one set of growing conditions could not necessarily be assumed to persist under another. By combining growth-phase analysis with direct evaluation of biomass and structural responses across contrasting environments, this study provides a field-relevant framework for assessing the performance of growth-modulating treatments in perennial bioenergy crops.

Such integrative assessment advances experimental optimisation towards field-relevant performance, a prerequisite for deploying growth-modulating strategies in climate-oriented perennial bioenergy systems.

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Supplementary materials

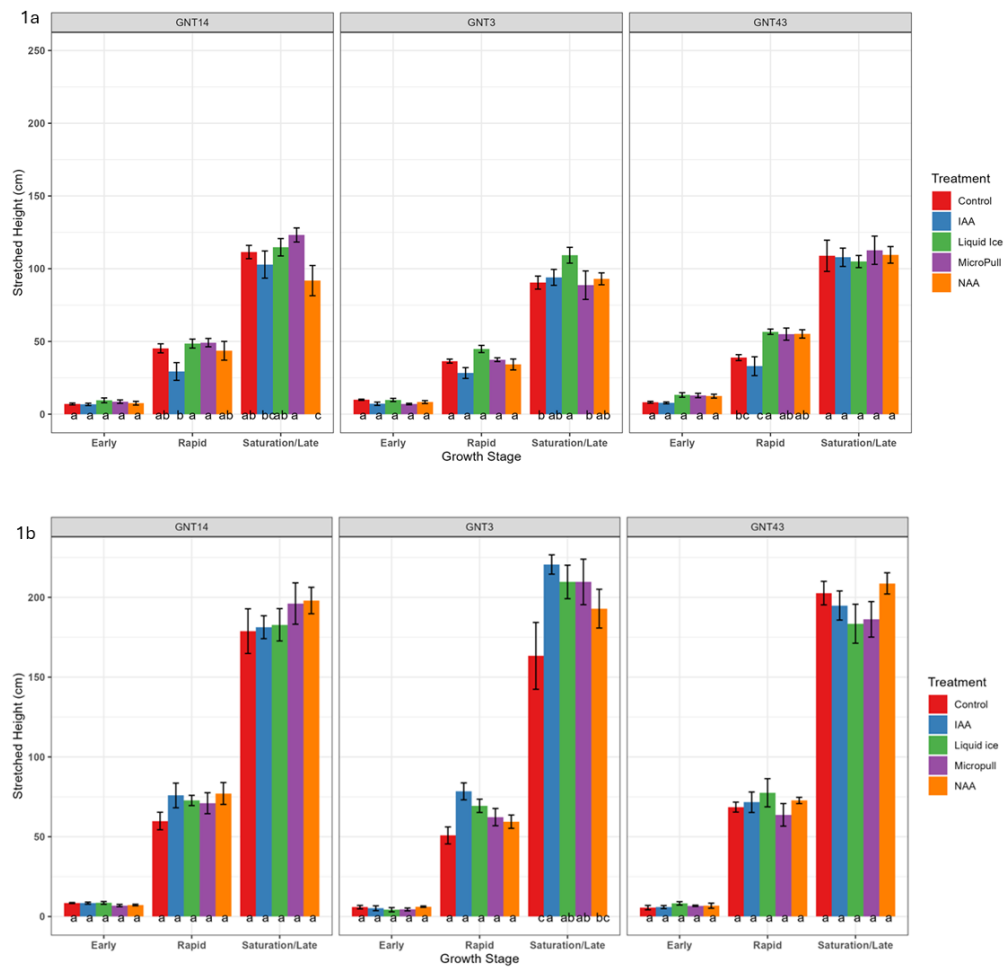


Figure S1. Developmental growth phases of stretched height. Stretched height (cm) partitioned into early, rapid elongation, and saturation phases for three *Miscanthus* hybrids under five PGR treatments. (a) Pot experiment (Year 1). (b) Field experiment (Year 1). Bars represent means $\pm$ SE (field $n = 4$, pot $n = 6$). Growth phases were defined by days after planting as described in Materials and Methods.

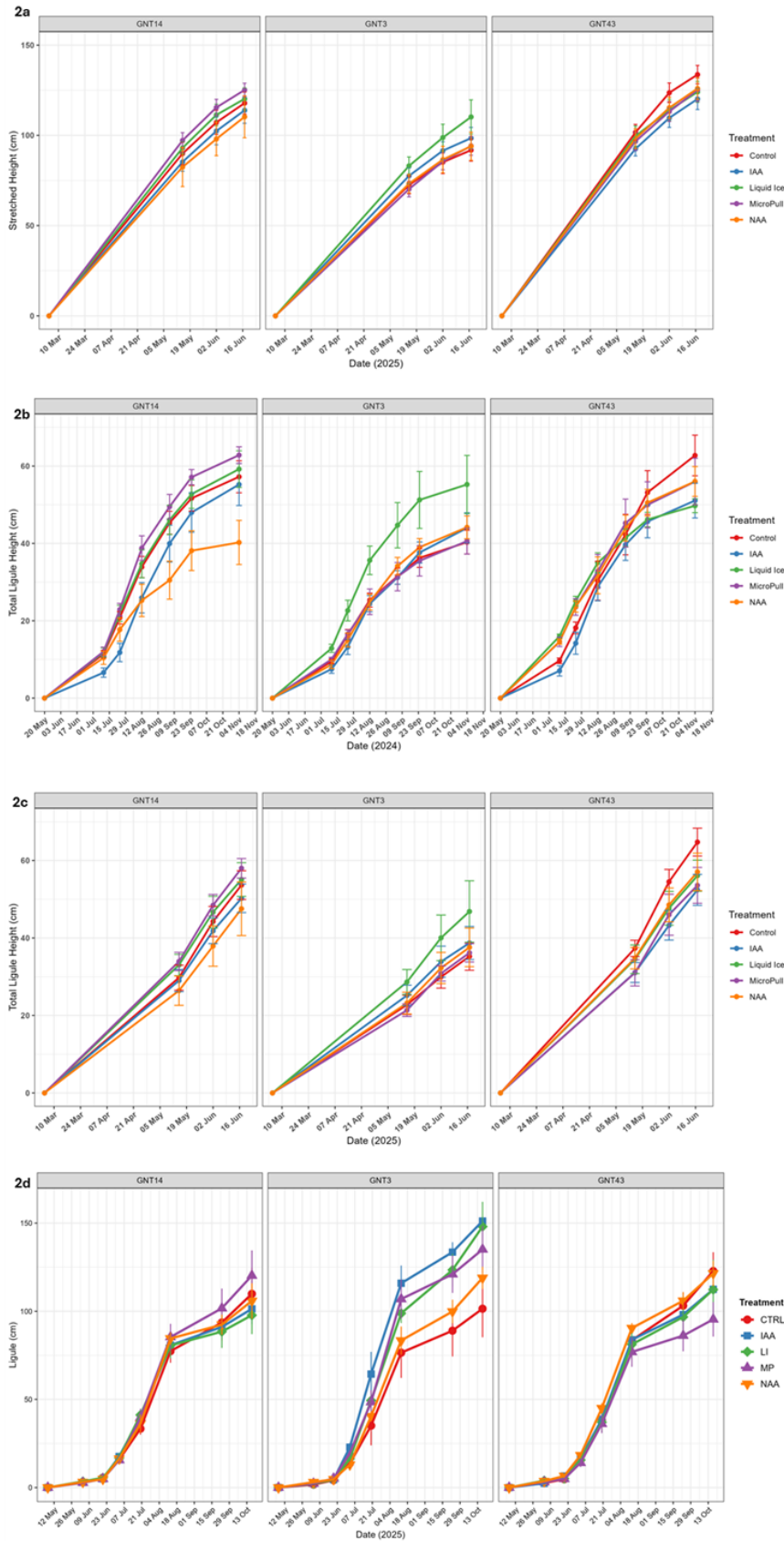


Figure S2. Extended structural metrics across seasons and environments.(a) Stretched height trajectories during Year 2 in pots.(b) Ligule height (Year 1, pots).(c) Ligule height (Year 2, pots).(d) Ligule height (Year 1, field).Points represent means $\pm$ SE across measurement dates (field $n = 4$, pot $n = 6$). Ligule height was measured as the distance from soil surface to the uppermost visible ligule and used as an indicator of structural stem elongation.

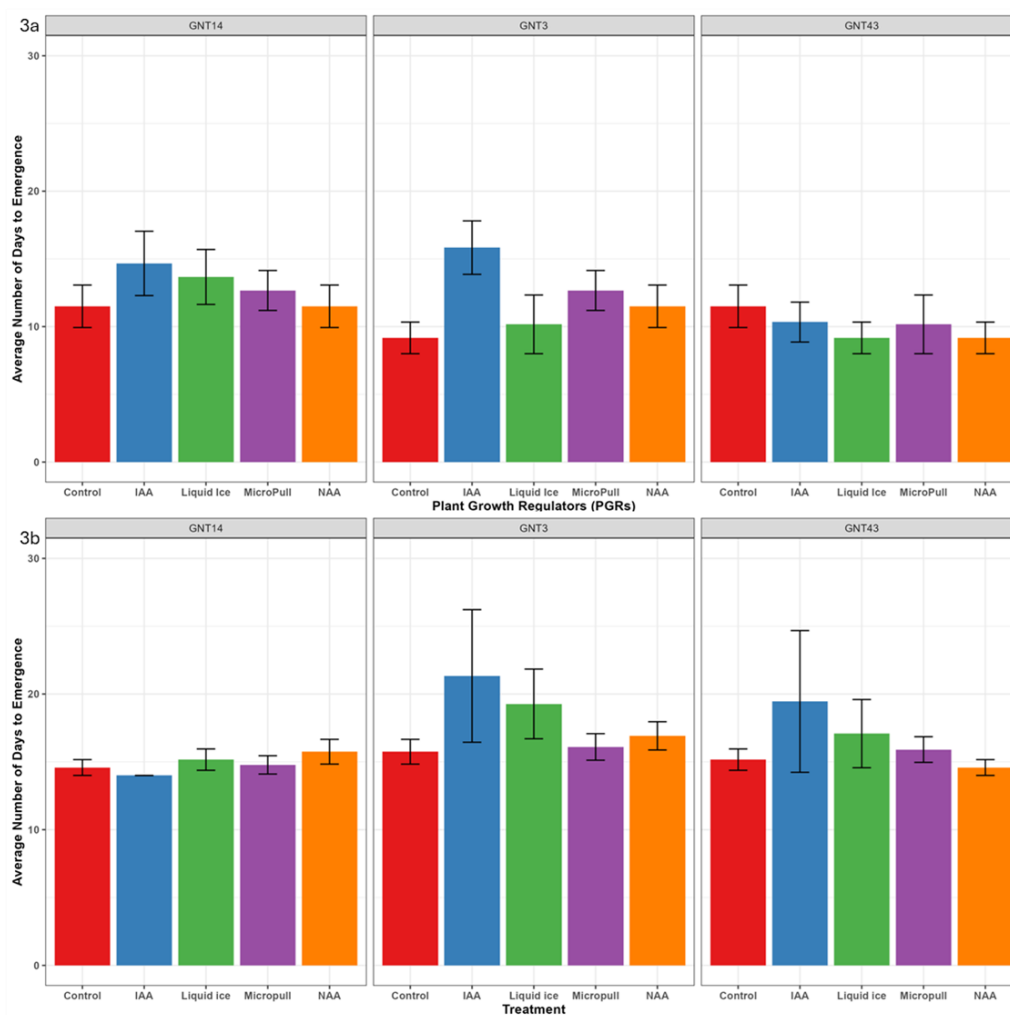


Figure S3. Emergence timing across environments. (a) Days to emergence in Year 1 pots. (b) Days to emergence in the field. Bars represent mean number of days to emergence $\pm$ SE for each hybrid $\times$ treatment combination (field $n = 4$, pot $n = 6$).

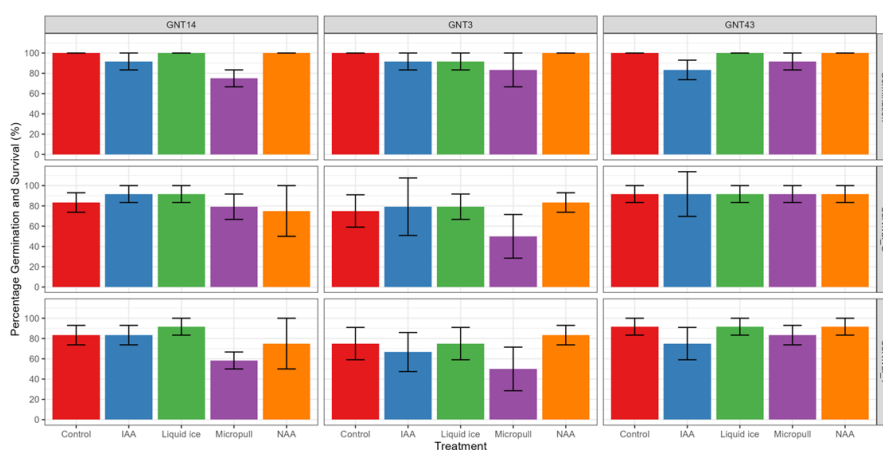


Figure S4. Germination and survival percentages in the field. (A) Percentage germination relative to total planted. (B) Survival_S: percentage surviving relative to total germinated. (C) Survival_T: percentage surviving relative to total planted. Bars represent means $\pm$ SE for each hybrid $\times$ treatment combination ($n = 4$).

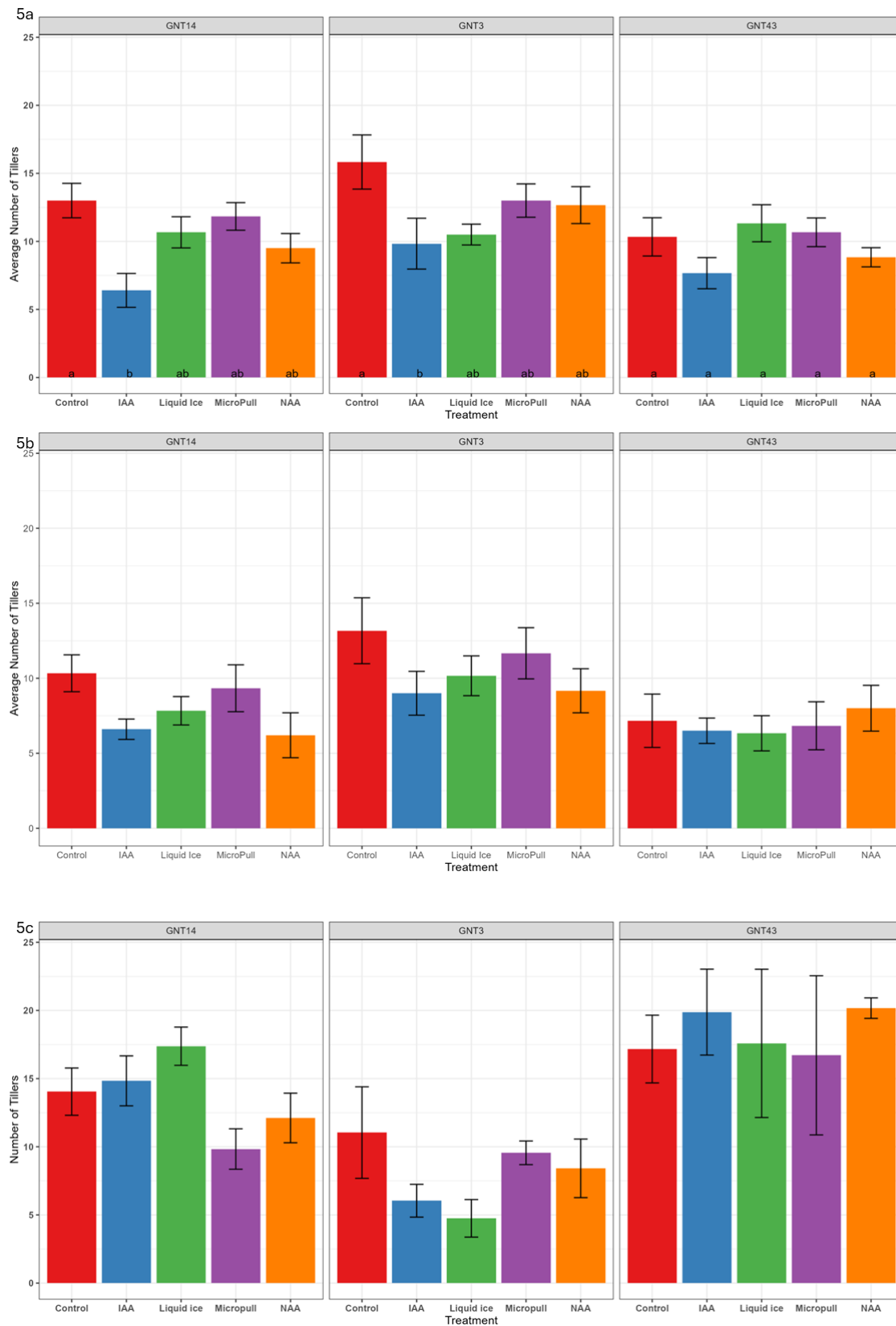


Figure S5. Tiller number across environments and seasons. (a) Tiller number in pots (Year 1). (b) Tiller number in pots (Year 2). (c) Tiller number in the field (Year 1). Bars represent means $\pm$ SE (field $n = 4$, pot $n = 6$). Tiller counts were recorded at peak vegetative development prior to harvest.

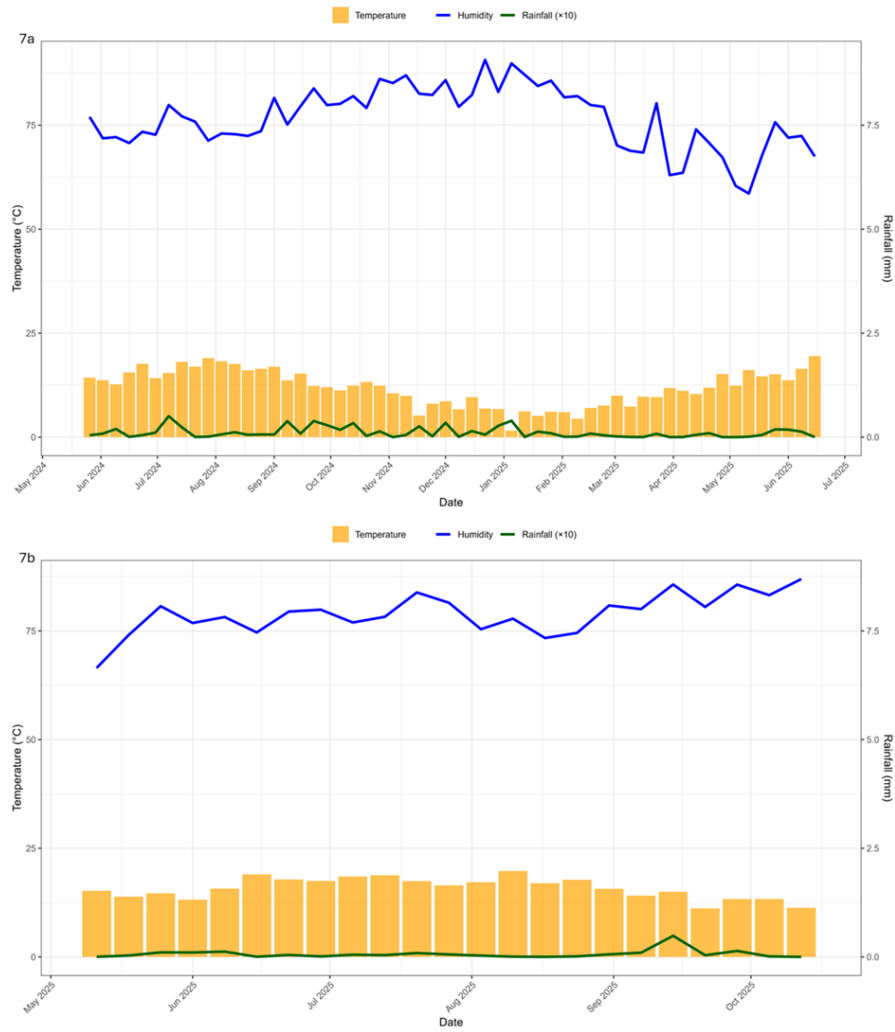


Figure S6. Weekly averages of temperature, humidity, and rainfall recorded at a.) pot site between May 2024 and June 2025, b.) field site between May 2025 and October 2025. Temperature is shown as bars, while humidity and rainfall (scaled $\times 10$) are shown as lines to illustrate seasonal patterns and variability across the study period.

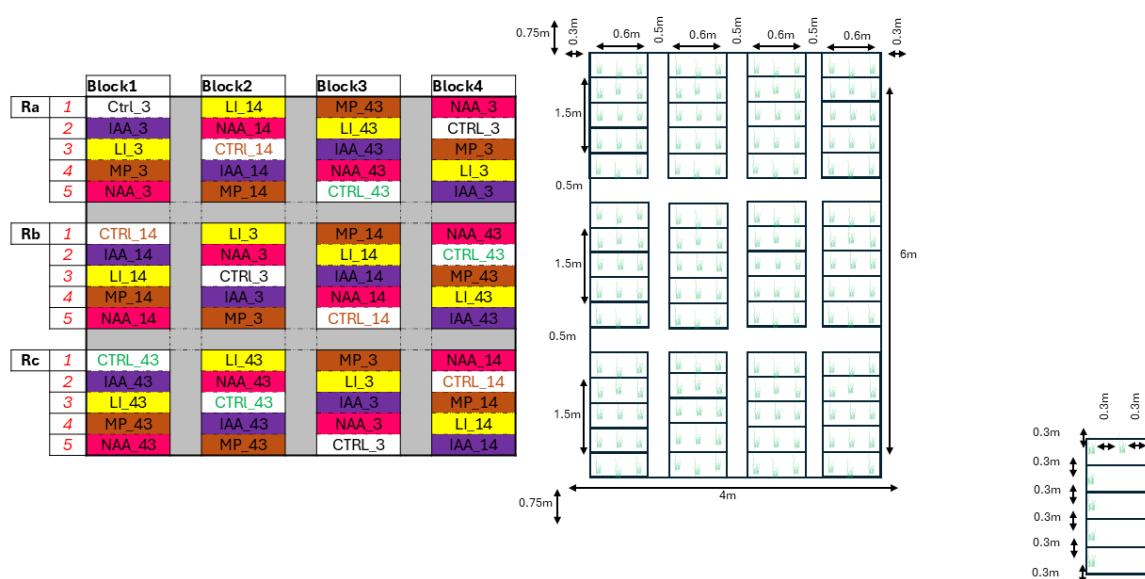


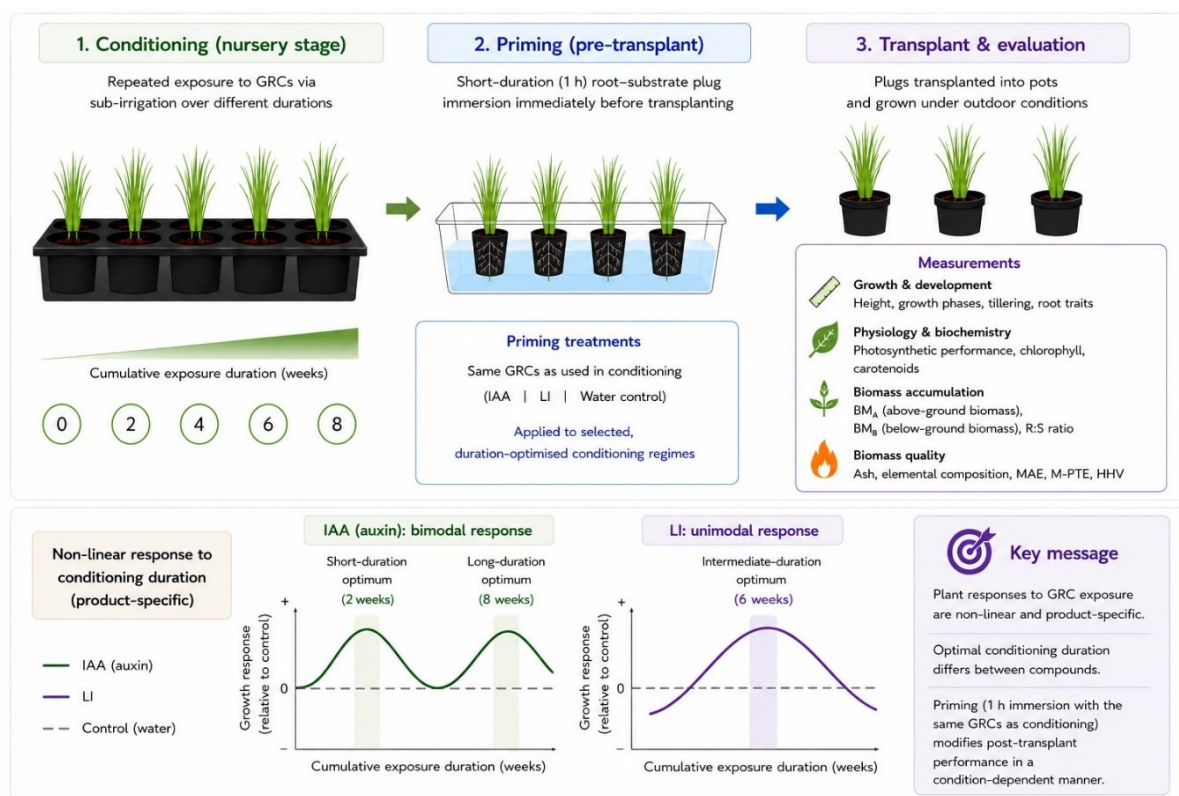
Figure S7. Experimental field layout and treatment allocation. Schematic representation of the randomised complete block design used in the field experiment. The left panel shows the allocation of treatment × hybrid combinations across four blocks, with each block subdivided into three replicate rows (Ra–Rc). Treatments include Control (CTRL), indole-3-acetic acid (IAA), liquid ice (LI), MicroPull (MP), and naphthaleneacetic acid (NAA), applied to three *Miscanthus* hybrids (GNT3, GNT14, GNT43). The right panel illustrates the spatial arrangement of plots, including plant spacing (0.3 m within plots), inter-plot spacing (0.5 m), and perimeter buffer zones (0.3 m) planted with *Miscanthus* to minimise edge effects. Plot and block dimensions are indicated.

Supplementary Table S1. Analysis of variance (ANOVA) results for structural growth phases, biomass accumulation, compositional traits, and establishment metrics across pot and field environments (Values shown are F-statistics and associated p-values. “–” indicates traits not measured in that environment).

Parameter	Effect	Pot Y1		Pot Y2		Field Y1	
		F	P	F	P	F	P
Early growth phase	Treatment	0.299	0.878	-	-	0.008	0.100
	Hybrid	0.696	0.500	-	-	0.218	0.804
	Treatment × Hybrid	0.131	0.998	-	-	0.024	1.000
Rapid growth phase	Treatment	9.384	0.000	-	-	2.419	0.052
	Hybrid	8.986	0.000	-	-	2.188	0.116
	Treatment × Hybrid	1.114	0.355	-	-	0.288	0.969
Late growth phase	Treatment	3.420	0.010	-	-	3.339	0.012
	Hybrid	16.405	0.000	-	-	2.085	0.128
	Treatment × Hybrid	3.818	0.000	-	-	4.294	0.000
Dry above biomass	Treatment	12.951	0.000	3.538	0.011	0.700	0.596
	Hybrid	3.951	0.024	8.635	0.000	9.025	0.001
	Treatment × Hybrid	4.932	0.000	2.160	0.041	1.066	0.404
Dry below biomass	Treatment	-	-	5.600	0.001	-	-
	Hybrid	-	-	14.609	0.000	-	-
	Treatment × Hybrid	-	-	4.054	0.001	-	-
HHV	Treatment	11.816	0.000	5.650	0.000	-	-
	Hybrid	0.517	0.598	0.207	0.814	-	-
	Treatment × Hybrid	1.947	0.063	3.056	0.004	-	-
Ash Content	Treatment	1.228	0.305	1.228	0.305	0.698	0.598
	Hybrid	0.907	0.407	0.907	0.407	2.221	0.121
	Treatment × Hybrid	0.590	0.784	0.590	0.784	0.316	0.955
Growth Rate	Treatment	47.416	0.000	1.431	0.224	9.418	0.000
	Hybrid	111.350	0.000	57.647	0.000	46.297	0.000
	Treatment × Hybrid	14.299	0.000	3.840	0.000	38.628	0.000
MAE	Treatment	21.506	0.000	1.342	0.266	0.044	0.996
	Hybrid	17.100	0.000	40.517	0.000	0.096	0.909
	Treatment × Hybrid	20.432	0.000	10.425	0.000	0.021	1.000
M-PTE	Treatment	19.443	0.000	0.716	0.585	0.053	0.994
	Hybrid	17.182	0.000	41.990	0.000	0.078	0.925
	Treatment × Hybrid	20.197	0.000	11.453	0.000	0.087	1.000
Carbon	Treatment	0.329	0.858	0.951	0.439	0.730	0.574
	Hybrid	0.553	0.577	0.638	0.531	0.892	0.413
	Treatment × Hybrid	1.633	0.127	0.803	0.602	0.920	0.503
Nitrogen	Treatment	4.019	0.005	0.029	0.998	2.894	0.026
	Hybrid	2.954	0.057	0.029	0.971	9.329	0.000
	Treatment × Hybrid	2.959	0.006	0.095	0.999	0.802	0.603
C:N ratio	Treatment	0.606	0.659	0.606	0.659	1.265	0.298
	Hybrid	3.093	0.051	3.093	0.051	7.586	0.002
	Treatment × Hybrid	1.362	0.225	1.362	0.225	0.566	0.800
Days to emergence	Treatment	1.553	0.196	-	-	0.997	0.411
	Hybrid	3.332	0.041	-	-	2.234	0.111
	Treatment × Hybrid	0.867	0.548	-	-	0.473	0.874
Tiller number	Treatment	5.320	0.000	1.932	0.115	0.203	0.935
	Hybrid	2.005	0.135	8.529	0.001	17.332	0.000
	Treatment × Hybrid	0.766	0.633	0.598	0.777	0.927	0.506
%Regrowth	Treatment	-	-	-	-	1.154	0.334
	Hybrid	-	-	-	-	0.032	0.969
	Treatment × Hybrid	-	-	-	-	0.192	0.992
Survival_G	Treatment	-	-	-	-	0.669	0.615
	Hybrid	-	-	-	-	2.941	0.056
	Treatment × Hybrid	-	-	-	-	0.477	0.871
Survival_T	Treatment	-	-	-	-	1.715	0.150
	Hybrid	-	-	-	-	2.404	0.094
	Treatment × Hybrid	-	-	-	-	0.441	0.895

Chapter 5

Growth-Regulating Compound Conditioning and Priming Improve Establishment and Biomass Quality in Seed-Derived *Miscanthus*.



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ABSTRACT

Seed-derived *Miscanthus* plug systems offer a scalable alternative to rhizome propagation but remain constrained by inconsistent establishment and suboptimal early growth. This study evaluated whether growth-regulating compounds (GRC), including an auxin (indole-3-acetic acid; IAA) and a biostimulant (Liquid Ice; LI), could enhance seed-plug development through conditioning duration and subsequent pre-transplant priming. In a 14-week nursery experiment, cumulative exposure duration (2–8 weeks) produced non-linear and product-specific growth responses. IAA exhibited a bimodal pattern, with short (IAA-2 weeks) and prolonged (IAA-8 weeks) exposure enhancing rapid-phase growth and biomass accumulation, while LI showed a unimodal response with optimal performance at intermediate exposure (LI-6 weeks). Selected conditioning regimes were evaluated post-transplant, where priming responses depended strongly on prior conditioning history. IAA priming enhanced growth, biomass accumulation, and photosynthetic electron transport, whereas LI priming reduced performance relative to conditioning alone. Across treatments, biomass gains were achieved without deterioration in key quality traits, including ash content and higher heating value. These findings demonstrate that GRC response in *Miscanthus* plugs are highly context-dependent and highlight the importance of optimising both exposure duration and priming strategy to improve establishment and biomass productivity in seed-derived systems.

Keywords: *Miscanthus*, plug establishment, priming, conditioning, plant growth regulators, biostimulants, biomass quality, bioenergy.

INTRODUCTION

Achieving global net-zero targets will require a substantial expansion of sustainable biomass resources capable of supplying low-carbon feedstocks for heat, electricity, and advanced biofuels (Lewandowski *et al.*, 2000; Heaton *et al.*, 2008; Clifton-Brown *et al.*, 2017). Among dedicated lignocellulosic crops, *Miscanthus* has emerged as one of the leading candidates due to its high biomass productivity, efficient resource-use characteristics, and favourable life-cycle greenhouse gas balance across diverse temperate environments (Heaton *et al.*, 2008; Lewandowski *et al.*, 2016; Clifton-Brown *et al.*, 2019). As a perennial C₄ grass, *Miscanthus* combines high radiation-use efficiency and photosynthetic capacity with relatively low fertiliser requirements and the ability to maintain productivity on marginal or lower-input land (Heaton *et al.*, 2008, Corinzia *et al.*, 2023; Awty-carroll *et al.*, 2026). These attributes have positioned *Miscanthus* as a strategic crop within European and global decarbonisation frameworks (Clifton-Brown *et al.*, 2019).

Despite this recognised potential, commercial cultivation remains limited. In Europe and the United Kingdom, the cultivated area remains far below projected land availability and policy-driven expansion targets (Lewandowski *et al.*, 2016; von Hellfeld *et al.*, 2022). A major contributor to this gap is the persistent challenge of reliable and cost-effective establishment. The first growing season represents the highest-risk stage of the production cycle, where poor establishment can reduce long-term productivity or result in complete stand failure (Clifton-Brown *et al.*, 2017; Hastings *et al.*, 2017). Establishment failures disproportionately increase production costs by delaying canopy closure, reducing first-year biomass accumulation, and necessitating replanting (von Cossel *et al.*, 2019).

Commercial *Miscanthus* has traditionally been established using rhizome propagation. Although rhizomes provide robust early growth due to stored reserves and pre-formed meristems, they are expensive to produce, difficult to store and transport, and labour-intensive to plant (Clifton-Brown *et al.*, 2007; 2017). Consequently, breeding and agronomic innovation have increasingly focused on seed-derived hybrids and plug-plant systems, which offer advantages in scalability, mechanisation, and genetic improvement (Clifton-Brown *et al.*, 2019). Seed-derived plugs enable uniform early development under

controlled nursery conditions before field transplantation, potentially reducing propagation costs. However, seedlings and young plugs often exhibit slower early growth, less developed root systems, and greater sensitivity to environmental transition compared with rhizome-established plants (Atkinson, 2009; Wu *et al.*, 2022; Ashman *et al.*, 2023). These limitations can impair anchorage, resource acquisition, overwinter survival, and second-year productivity, which are key determinants of stand persistence and long-term yield.

Improving early-stage development in plug systems therefore represents a critical opportunity to enhance establishment reliability and accelerate adoption. Growth-regulating compounds (GRC), including plant growth regulators (PGR) and biostimulants (BS), are increasingly being explored as low-input tools for manipulating early developmental processes and enhancing stress resilience during crop establishment (Obaje *et al.*, 2026). In the context of plug production, two operational approaches can be applied. First, conditioning; this refers to the repeated application of growth-regulating compounds during nursery growth to influence developmental trajectories over time, consistent with approaches used to manipulate seedling vigour and stress tolerance during propagation (Colla *et al.*, 2014; Rouphael & Colla, 2020). Second, priming refers to a short-duration treatment applied immediately prior to transplanting, intended to transiently modify physiological state at the point of environmental transition, analogous to pre-sowing or pre-transplant priming strategies widely reported in various crop systems (Paparella *et al.*, 2015; Van Oosten *et al.*, 2017). While both approaches aim to improve establishment, conditioning acts cumulatively during early development, whereas priming targets immediate post-transplant responses.

Among PGR, auxins regulate root initiation, vascular differentiation, cell elongation, and tropic responses (Davies, 2010). Exogenous auxins are widely used in horticulture and forestry to improve rooting and transplant success, but their effects are highly dose-, duration-, and stage-dependent. While low concentrations or short exposures may stimulate growth, excessive or prolonged exposure can inhibit leaf expansion, suppress biomass accumulation, and disrupt endogenous hormonal balance (Keller *et al.*, 2004; Kurepa *et al.*, 2018; Li and Chen, 2023). In perennial grasses and bioenergy crops such as *Miscanthus*, this context dependency limits the reliability of auxin-based interventions.

Biostimulants represent a broader and more heterogeneous class of GRC. Although diverse in composition, BS products are generally defined by their ability to enhance nutrient-use efficiency, stress tolerance, or crop performance independent of direct nutrient supply (du Jardin, 2015; Rouphael & Colla, 2020). Their effects may arise through hormonal signalling, osmotic regulation, improved nutrient assimilation, or enhanced antioxidant activity. Complex formulations containing humic substances, peptides, polysaccharides, phenolics, and betaines can influence membrane transport, nitrogen metabolism, and redox homeostasis through multi-component biochemical interactions (Calvo *et al.*, 2014; Yakhin *et al.*, 2017; Rouphael & Colla, 2020). However, responses are often inconsistent across products, environments, and genotypes, and their mechanisms remain less well characterised than classical phytohormones.

A key challenge in applying GRC during plug production is that plant responses are rarely linear and often depend on cumulative exposure duration, product chemistry, and developmental timing (Woodward & Bartel, 2005; Sauer *et al.*, 2013; Yakhin *et al.*, 2017). For many products, including auxin-based regulators and complex BS formulations, the exposure thresholds that maximise early growth while avoiding inhibitory effects remain unclear. At the same time, short-duration priming treatments applied immediately before transplanting may offer a practical means of enhancing establishment without increasing greenhouse residence time (Paparella *et al.*, 2015; Van Oosten *et al.*, 2017; Rouphael & Colla, 2020), but their effectiveness in *Miscanthus* plug systems has not been evaluated. Addressing these uncertainties requires determining the cumulative exposure durations at which conditioning is beneficial and assessing whether brief pre-transplant priming can further improve early performance under operationally realistic timeframes.

The present study addresses these knowledge gaps by integrating a conditioning-duration optimisation experiment with a subsequent priming experiment using selected, duration-optimised conditioning regimes. Specifically, the authors investigated whether cumulative exposure duration to selected PGR and BS treatments influences early growth and developmental trajectories in seed-derived *Miscanthus* plugs, and whether these pre-conditioned physiological states alter responsiveness to a brief pre-transplant priming treatment. We hypothesised that (i) responses to GRC exposure would be non-

linear and aligned with specific developmental phases, and (ii) conditioning would modify post-transplant growth dynamics, biomass accumulation, physiological performance, and biomass quality, with priming acting as a secondary modifier of these responses rather than a consistently additive treatment. By integrating morphological, physiological, biochemical, and biomass-quality analyses, this study provides insight and practical guidance for improving the scalability and reliability of seed-derived *Miscanthus* plug production systems.

MATERIALS AND METHODS

Experimental overview

This study comprised two sequential experiments designed to evaluate the effects of growth-regulating compounds (GRC), including IAA (Indole-3-acetic acid) a plant growth regulator and LI (Liquid ice) a biostimulant, on nursery-stage plug development and subsequent post-transplant performance of seed-derived *Miscanthus*. In Experiment 1, a nursery-stage conditioning trial was conducted to determine how cumulative exposure duration to the GRC influenced early growth trajectories, biomass accumulation, and root development during plug production. Based on the outcome of this threshold experiment a short-duration pre-transplant priming treatment was applied to pre-selected treatment for Experiment 2. Treatment structures for both experimental stages are summarised in Table 1.

Table 1. Summary of treatment regimes applied during nursery-stage conditioning (Experiment 1) and post-transplant priming experiments (Experiment 2). Exposure duration refers to the cumulative period of compound application during nursery-stage plug. Priming duration refers to the 1-h pre-transplant soaking treatment applied immediately before transplanting.

Stage	Treatment code	Compound	Concentration	Exposure duration	Priming duration
Nursery	IAA_2	IAA	2 mL L ⁻¹	2 weeks	-
	IAA_4	IAA		4 weeks	-
	IAA_6	IAA		6 weeks	-
	IAA_8	IAA		8 weeks	-
	LI_2	LI	0.5 mL L ⁻¹	2 weeks	-
	LI_4	LI		4 weeks	-
	LI_6	LI		6 weeks	-
	LI_8	LI		8 weeks	-
	CTRL_0	Water		0 weeks	-
Transplant	IAA_0	IAA	-	-	-
	IAA_1	IAA	2 mL L ⁻¹	-	1 hour
	LI_0	LI	-	-	-
	LI_1	LI	0.5 mL L ⁻¹	-	1 hour
	CTRL	Water	-	-	-

IAA = indole-3-acetic acid; LI = Liquid Ice, a commercial biostimulant. Numeric suffixes in nursery-stage treatment codes indicate the duration of compound exposure in weeks prior to withdrawal (e.g., IAA_2 = 2 weeks of IAA application). In transplant-stage treatment codes, suffixes indicate absence (0) or presence (1) of a 1-h pre-transplant priming treatment. Exposure duration refers to cumulative application during nursery-stage plug development; priming duration refers to soaking immediately prior to transplanting.

Plant material and growth conditions

The plant material used in experiment 1 and 2 was a seed-derived *Miscanthus* hybrid (*Miscanthus sacchariflorus* × *Miscanthus sinensis*), GNT43 ('Aphrodite'), developed through the Giant-Link *Miscanthus* breeding programme and supplied by Terravesta Ltd. This genotype was selected due to its commercial relevance and consistent performance in previous nursery-stage experiments (Wu *et al.*, 2022; Obaje *et al.*, 2026).

The nursery-stage conditioning experiment was conducted in the research glasshouse of Liverpool John Moores University, Liverpool, UK (53°24'45.1"N, 2°58'47.7"W), from 19th December 2024 (when seeds were sown) to 28th March 2025 (when final data was collected on the grown plugs). During this period, average glasshouse temperature and relative humidity were approximately 20 ± 5 °C and 50 ± 10 %, respectively. Because this experiment was conducted during winter and early spring when natural irradiance was limited, a 16/8-h light/dark cycle was maintained using supplementary Kroptek KP4 broad-spectrum LED lighting (Kroptek Ltd, UK).

Seeds were sown in 12-celled propagation trays (Mixceco) containing Levington Multipurpose Compost (Evergreen Garden Care, UK). Three seeds were sown per cell and, following successful germination, were thinned to one seedling per cell. Transparent

tray lids supplied with the trays were maintained for two weeks after sowing to sustain the humid environment required for *Miscanthus* germination.

The post-transplant experiment was conducted between 4th April 2025 and 1st September 2025 outdoors around the glasshouse mentioned in experiment 1. Plants were maintained under ambient environmental conditions in pots placed in trays lined with capillary matting, allowing substrate moisture to be maintained through a controlled irrigation regime. Average temperature and relative humidity during this period were approximately 25 °C and 70 %, respectively and this was measured using the Ecowitt WS3X02 weather station (Ecowitt, Shenzhen, China). Weekly mean environmental conditions during the post-transplant period are presented in Supplementary Figure S8.

Experiment 1: nursery-stage conditioning-duration optimisation

The first experiment investigated the effect of cumulative exposure duration to two GRC during nursery-stage plug development: indole-3-acetic acid (IAA; The Plant Growth Company, UK), an auxin-based PGR, and Liquid Ice (LI; Evoponic Ltd, UK), a commercial BS formulation. These compounds were selected based on prior preliminary screening and nursery-stage studies (Obaje *et al.*, 2026). IAA was applied at 2 mL L⁻¹ and LI at 0.5 mL L⁻¹ (Table 1).

Each compound was applied for four cumulative exposure durations of 2, 4, 6, or 8 weeks from the start of the experiment (19th December 2024), alongside an untreated control receiving only water throughout (CTRL_0), resulting in nine treatments in total (Table 1). Treatment commenced on the day of sowing. Following cessation of treatment at the designated durations, plugs received only water until final harvest.

The experiment followed a randomised complete block design (RCBD) with five independent trays per treatment, each tray serving as a block. Each tray contained 12 cells, of which six predetermined plants were selected as subsamples for repeated measurement. Because plants within a tray shared the same rooting environment and irrigation regime, these six plants were not treated as independent replicates. Instead, measurements were averaged to produce a single block-level mean for statistical analysis. Consequently, each treatment had five independent biological replicates (n = 5), and the experiment comprised 270 monitored plants.

Treatments were delivered via sub-irrigation by adding treatment solution to drain trays beneath each propagation tray, following the approach described by Obaje *et al.* (2026). During early establishment, irrigation (either treatment solution or water, depending on treatment stage) was applied approximately once weekly (~250 mL per tray). As plants increased in size and substrate moisture demand rose, irrigation frequency was increased to twice weekly while maintaining the assigned treatment schedule across all exposure groups.

Nursery-stage growth and biomass measurements

Growth was monitored weekly throughout the experiment. Stretched height, defined as the distance from the substrate surface to the tip of the tallest extended leaf (Nunn *et al.*, 2017), was measured using a metric ruler.

Based on observed growth trajectories, plant development was partitioned into three biologically defined phases expressed as weeks after sowing (WAS): establishment (0–6 WAS), rapid elongation (6–9 WAS), and late growth (>9 WAS). These boundaries corresponded to clear shifts in growth rate (Table 2) and allowed treatment effects to be examined within biologically meaningful developmental windows.

Growth rate (GR) was calculated between the minimum (H_{min}) or maximum (H_{max}) height:

$$GR = \frac{(H_{max} - H_{min})}{t}$$

Where the time interval (t) corresponded to the duration of the respective phase or entire period. Phase-specific growth rates were derived for the establishment phase (R_{ep}), rapid phase (R_{rp}), and late phase (R_{lp}), in addition to an overall growth rate (growth_rate.overall) across the full trajectory.

At final harvest, plants were separated into above-ground biomass (BM_A) and below-ground biomass (BM_B), weighed fresh, and oven-dried at 90 °C to constant weight before reweighing, following standard biomass determination procedures (Sluiter *et al.*, 2008). Biomass allocation was quantified as the root-to-shoot ratio ($R:S = BM_B / BM_A$). Percentage changes in final height, BM_A , and BM_B relative to the untreated control were also calculated.

Root development was evaluated using both quantitative and semi-quantitative approaches. Root biomass was quantified as BM_B. Representative root images were collected to document morphological differences among treatments (Supplementary Figure S2). Root density was assessed using a three-point visual scoring system based on root compactness and branching intensity, where scores of 1 to 3 represented less dense to dense root systems, respectively. To improve scoring consistency and reliability, visual assessments were repeated twice at two-week intervals by the same assessor using the root images. Representative shoot images were also documented (Supplementary Figure S3). Additional shoot architecture was assessed by counting tillers and visible basal buds at harvest.

Associations among growth and biomass traits were examined using Spearman rank correlations (Supplementary Figure S1).

Experiment 2: post-conditioning priming and transplant experiment

Based on the outcomes of Experiment 1, plugs in treatments IAA_2 and LI_6 were selected for further evaluation. Although IAA_8 also produced strong responses, IAA_2 was selected because it provided comparable benefits under a substantially shorter conditioning period, making it more operationally and economically feasible for commercial plug production.

Five treatments were evaluated: untreated control (CTRL), IAA-conditioned plugs without priming (IAA_0), IAA-conditioned plugs subjected to 1-h priming (IAA_1), LI-conditioned plugs without priming (LI_0), and LI-conditioned plugs subjected to 1-h priming (LI_1) (Table 1).

All plugs were transplanted on the same day. Prior to transplanting, all plugs were cut back to approximately 3 cm. Thereafter, a subset of the IAA- and LI-conditioned plugs (IAA_1 and LI_1) received a short priming treatment consisting of complete submergence of the root-substrate plug in the corresponding treatment solution at the same concentration used during conditioning. Priming duration was not formally optimised; rather, a 1-h exposure period was selected based on informal preliminary observations indicating that this duration was sufficient for complete saturation of the root bulk, although further optimisation of priming duration may improve treatment efficacy. The

intention was to use the root–substrate plug as a temporary carrier matrix for rapid treatment uptake prior to transplanting. Control plants and corresponding non-primed subsets were not subjected to the priming treatment and received no additional PGR, biostimulant, or water application prior to transplanting.

Following priming, plants were transplanted into 5 L rose pots (Muddy Hands Ltd. UK) filled with approximately 4 L of Wickes Multi-Purpose Topsoil (Wickes plc, Watford, UK). The experiment followed a completely randomised design (CRD) comprising five treatments with five biological replicates each ($n = 5$; 25 plants total).

Following transplanting, pots were placed in trays lined with capillary matting as described earlier. Water was supplied to the trays automatically three times daily to facilitate passive uptake through the pot base. In addition, plants were aerially watered approximately once weekly to ensure uniform wetting of the upper soil profile.

Post-transplant morphological and physiological measurements

Post-transplant development was monitored over 150 days after transplanting (DAP). Stretched height and ligule height were measured weekly for the first 40 days and thereafter bi-weekly until the penultimate measurement. Leaf and tiller numbers were recorded manually.

Regrowth was monitored daily, and time to 50% regrowth (T_{50}) was defined as the number of days required for at least half of the plants within a treatment to show visible regrowth (emergence of a new green shoot).

Growth was additionally summarised by developmental stage to facilitate biologically meaningful comparisons across distinct phases of post-transplant establishment and canopy expansion. Based on observed inflection points in the stretched-height growth trajectory, three stages were defined: early establishment (≤ 30 DAP), characterised by initial acclimation and limited elongation; rapid growth (31–80 DAP), corresponding to the period of maximum elongation and canopy expansion; and saturation/late growth (> 80 DAP), during which height increase slowed and approached a plateau.

Photosynthetic performance was assessed using a MultispeQ device (PhotosynQ Inc., USA) running the RIDES 2.0 protocol. Parameters extracted included linear electron flow

(LEF), quantum yield of PSII (Φ_{II}), and non-photochemical quenching (Φ_{NPQ}). Relative chlorophyll content was estimated using SPAD.

Pigment-related indices were measured using a handheld pigment meter (ADC BioScientific Ltd, UK), including anthocyanin index (AnthM), chlorophyll index (ChlM), flavonol index (FlvM), and nitrogen-flavonol index (NFI).

Biomass quality and biochemical analyses

At final harvest, plants were partitioned into above- and below-ground biomass and processed as described in experiment 1 to quantify biomass.

Determination of higher heating value

Higher heating value (HHV) of stem biomass was determined using an oxygen bomb calorimeter (Model 6050 compensated jacket calorimeter; Parr Instrument Company, USA). Following the manufacturer's instructions, the heat of combustion of dried samples was measured and converted to HHV following ASTM D5865 and ISO 18125 standard procedures.

Determination of ash content and elemental composition

Ash content was determined by combusting oven-dried stem samples in a furnace (Carbolite, UK) at 540 °C for 4 h. Samples were weighed before and after ashing, and ash content (%) was calculated on a dry-weight basis. Ash samples were subsequently analysed for elemental composition using X-ray fluorescence (XRF; Epsilon 1, Malvern Panalytical Ltd, UK). Elements were grouped into major ash-forming elements (MAE: K, Ca, Mg, Si, P, S, Cl) and minor and potentially toxic elements (M-PTE: Pb, Ni, Cr, Cu, Co, V), following Endriss *et al.* (2024). Full elemental profiles are provided in Supplementary Figure S6.

Determination of total organic carbon and total nitrogen

Following the manufacturer's instructions, approximately 100 mg of oven-dried samples were analysed for total organic carbon and total nitrogen using a Skalar PRIMACS™ SNC-100 analyser (Skalar Analytical B.V., Netherlands).

Determination of lutein and β -carotene concentrations

Carotenoids were extracted from plant tissue using an acetone-based protocol commonly applied for photosynthetic pigment analysis (Lichtenthaler, 1987). Approximately 200 mg of fresh leaf tissue from the last fully expanded leaf was weighed into 2 mL amber microcentrifuge tubes, snap-frozen in liquid nitrogen, and ground to a fine powder using a chilled mini pestle. Pigments were extracted by adding 0.5 mL acetone, followed by vigorous vortexing and centrifugation at maximum speed for 5 min. The supernatant was transferred to a clean amber tube, and the pellet was re-extracted with an additional 0.5 mL acetone using the same procedure. This extraction step was repeated until the pellet was no longer visibly green (up to 2 mL). Pigment extraction was performed under reduced-light conditions, with laboratory lights switched off and blinds closed, and samples and extracts were kept on ice during the extraction procedure to minimise pigment degradation. Combined supernatants were evaporated to dryness under a stream of nitrogen gas and reconstituted in 500 μ L acetone prior to HPLC analysis.

Trans-lutein and trans- β -carotene concentrations were determined by high-performance liquid chromatography (HPLC) using an Agilent 1260 system (Agilent Technologies, USA), following the approach described by Symonds *et al.* (2007) with minor modifications.

The mobile phase consisted of solvent A (methanol containing 0.1% trifluoroacetic acid, TFA) and solvent B (methyl tert-butyl ether). The gradient programme was adapted from Symonds *et al.* (2007) and applied as follows: 95% A / 5% B held from 1–10 min; increased to 85% A / 15% B at 11 min; ramped to 60% A / 40% B at 25 min; to 35% A / 65% B at 35 min; to 15% A / 85% B at 50 min; followed by 100% B at 55 min. The column was then re-equilibrated to initial conditions (95% A / 5% B) by 60 min and held until 65 min. The flow rate was set at 0.5 mL min⁻¹ and separation was performed on a 5 μ m, 250 $\times$ 4.6 mm YMC Pack C30 column (YMC Europe GmbH) combined with a guard column (SecurityGuard, Phenomenex, Macclesfield, UK) maintained at 30 °C.

Carotenoid identification was made using both retention time (t_R) and absorption spectrum (λ _{max} and spectral shape) in comparison to authentic standards of trans-lutein and trans- β -carotene (Sigma-Aldrich) dissolved in acetone. Stock solutions were

serially diluted to generate a five-point calibration range. Each dilution was injected under identical chromatographic conditions, and peak areas were plotted against standard concentration. Linear regression was used to generate calibration equations, which were applied to calculate carotenoid concentrations in plant samples. Calibration curves exhibited linear responses across the concentration range analysed ($R^2 \geq 0.99$). Representative calibration curves are provided in Supplementary Figure S7.

Statistical analysis

All statistical analyses were performed in R version 4.6.0 (R Core Team, 2025).

For both experiments, repeated measurements were analysed using linear mixed-effects models implemented with the `lmer()` function in the `lme4` package (Bates *et al.*, 2015), with treatment, time, and their interaction specified as fixed effects and block included as a random effect. Where significant treatment $\times$ time interactions were detected, estimated marginal means and Tukey-adjusted pairwise comparisons were obtained using the `emmeans` package (Lenth, 2025).

To evaluate groups of conceptually related response variables within a common analytical framework, for example physiological variables (LEF, Φ II, Φ NPQ, and SPAD), pigment-related variables (AnthM, ChlM, FlvM, NFI, and carotenoid concentrations), and yield variables (biomass and root-to-shoot ratio) were each reshaped into long format and analysed using separate mixed-effects models, with parameter included as an additional fixed effect. Parameter-specific effects were subsequently assessed using the `joint_tests()` function in the `emmeans` package.

Model assumptions were evaluated by inspection of residual and quantile–quantile (Q–Q) plots. Variables that did not satisfy assumptions of normality, including time to 50% regrowth (T50), were analysed using the Kruskal–Wallis test.

Associations among key growth and biomass variables were assessed using Spearman rank correlation coefficients computed with the `cor()` function and visualised using the `corrplot` package. Figures were produced using the `ggplot2` package, and data handling and statistical workflows were performed using base R and the tidyverse suite of packages. Unless otherwise stated, results are presented as means $\pm$ standard error.

RESULTS

Exposure duration and product type altered growth trajectories during plug development

Plant height increased progressively throughout the 14-week plug development period across all treatments, but the magnitude and timing of elongation responses varied substantially with both growth-regulating compound (GRC) type and exposure duration (Figure 1). Across treatments, growth followed three distinct developmental phases: an establishment phase (0 – 6 weeks), a rapid growth phase (6 – 9 weeks), and a late growth phase (≥ 9 weeks). During the establishment phase, differences among treatments were relatively modest, with most GRC-treated plants exhibiting only small numerical and non-statistically significant ($p > 0.05$) increase over the untreated control. Clear treatment-driven separation emerged during the rapid growth phase, where elongation rates increased sharply and became strongly dependent on both product type and cumulative exposure duration (Figure 1). In the late growth phase, elongation rates declined, with reduced variation between some treatments, although differences between treatments remained evident. This indicates that the principal effects of GRC exposure were expressed during the rapid stem elongation period, with some treatment differences persisting into later development.

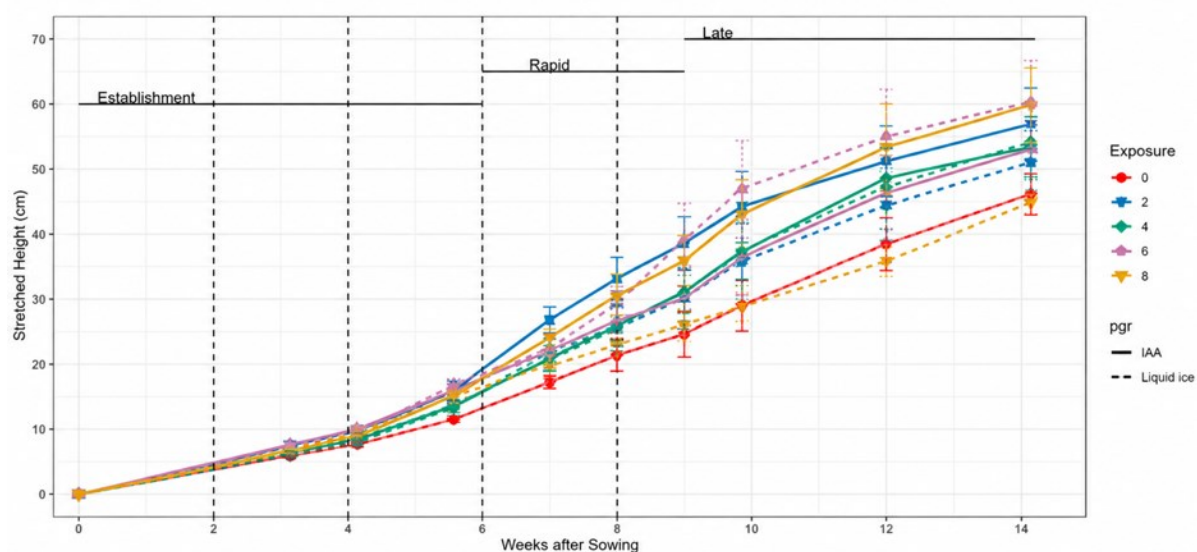


Figure 1. Stretched (maximum) height across growth phases under varying exposure duration and plant growth regulators. Line plot showing total plant height (cm) from 0–14 weeks after sowing under five exposure duration (0, 2, 4, 6, 8). Solid lines represent IAA treatments and dashed lines represent Liquid Ice treatments. Error bars indicate mean

± SE (n = 5). The horizontal solid line at the top marks the three predefined growth phases: Establishment (0–6 weeks), Rapid Growth (6–9 weeks), and Late Growth (≥ 9 weeks). The vertical dashed lines indicate the time points at which treatments were stopped for the relevant exposure levels.

Among IAA-treated plugs, responses were non-linear and appeared bimodal, with the greatest rapid growth phase elongation rates observed under both short and prolonged exposure durations. IAA_2 and IAA_8 produced rapid-phase growth rates of 5.89 ± 0.72 and 5.91 ± 0.70 cm week⁻¹, respectively, both significantly greater than the control (3.69 ± 0.70 cm week⁻¹) and intermediate IAA durations (Table 2). Intermediate IAA exposures produced more moderate gains, with IAA_4 and IAA_6 exhibiting rapid-phase growth rates of 5.12 ± 0.38 and 4.01 ± 0.59 cm week⁻¹, respectively. In contrast, Liquid Ice exhibited a unimodal response pattern, with LI_6 producing the highest rapid growth phase elongation rate of all treatments (6.88 ± 0.95 cm week⁻¹), significantly exceeding both the control and LI_8 ($p > 0.05$). Prolonged Liquid Ice exposure reduced performance markedly, with LI_8 exhibiting the lowest rapid-phase growth rate among treated plants (3.11 ± 0.18 cm week⁻¹), approaching control levels.

Table 2. Growth rates and percentage changes in final height, above-ground biomass (BM_A), and below-ground biomass (BM_B) under IAA and Liquid Ice treatments. [$R_{ep}/R_{rp}/R_{lp}$: early/rapid/late growth rate, H_{pc} : percentage changes in final height, $BM_{A,pc}$ and $BM_{B,pc}$: percentage changes in above and below ground biomass, respectively].

PGR	Exp	Treatment	R_{ep} (cm/week)	R_{rp} (cm/week)	R_{lp} (cm/week)	$H_{pc}(\%)$	$BM_{A,pc}(\%)$	$BM_{B,pc}(\%)$
Control	0	Control_0	2.31 ± 0.07^a	3.69 ± 0.70^{cd}	3.99 ± 0.13^a	-	-	-
IAA	2	IAA_2	3.49 ± 0.19^a	5.89 ± 0.72^{ab}	3.21 ± 0.15^a	+26	+223	+120
	4	IAA_4	2.88 ± 0.10^a	5.12 ± 0.38^{bc}	3.85 ± 0.21^a	+17	+53	+17
	6	IAA_6	3.42 ± 0.13^a	4.01 ± 0.59^{cd}	4.24 ± 0.20^a	+19	+51	+22
	8	IAA_8	3.53 ± 0.11^a	5.91 ± 0.70^{ab}	4.06 ± 0.11^a	+31	+264	+100
Liquid ice	2	Liquid ice_2	3.17 ± 0.11^a	4.80 ± 0.82^{bcd}	3.56 ± 0.20^a	+11	+38	-1
	4	Liquid ice_4	2.59 ± 0.21^a	4.60 ± 0.30^{bcd}	3.91 ± 0.11^a	+18	+24	+18
	6	Liquid ice_6	4.00 ± 0.13^a	6.88 ± 0.95^a	3.35 ± 0.16^a	+33	+197	+104
	8	Liquid ice_8	3.47 ± 0.15^a	3.11 ± 0.18^d	3.74 ± 0.09^a	-3	+40	+15

Growth rates are presented as mean ± SE (n = 5). Percentage change (pc) values express the proportional increase or decrease relative to the untreated control (Control_0). Letters following means indicate statistical groupings based on post-hoc comparisons, where treatments sharing a letter are not significantly different.

These differences in elongation dynamics were reflected in final plant height and overall treatment performance. Relative to the untreated control, final height increased by 26% under IAA_2, 31% under IAA_8, and 33% under LI_6, whereas LI_8 resulted in a slight reduction in final height (-3%) (Table 2). Across both products, treatments associated with enhanced rapid growth phase elongation also tended to exhibit greater total biomass accumulation at harvest. Collectively, these results demonstrate that plug

responses to GRC exposure were strongly product-specific and non-linear, with distinct duration optima depending on compound type and developmental timing.

Biomass accumulation and allocation responses across exposure durations

Patterns of biomass accumulation broadly mirrored the growth responses observed during plug development, with selected exposure durations producing substantial increases in both above- and below-ground biomass (Figure 2). Above-ground biomass differed significantly among treatments and was greatest under IAA_2, IAA_8, and LI_6. Relative to the untreated control, these treatments increased above-ground biomass by 223%, 264%, and 197%, respectively (Table 2). Intermediate exposure durations produced smaller gains, with IAA_4 and IAA_6 increasing above-ground biomass by 53% and 51%, respectively, while LI_2, LI_4, and LI_8 produced more modest responses ranging from 24–40%. These patterns were consistent with the rapid growth phase elongation responses observed in Figure 1, suggesting that enhanced biomass accumulation was closely associated with increased growth during the mid-developmental phase.

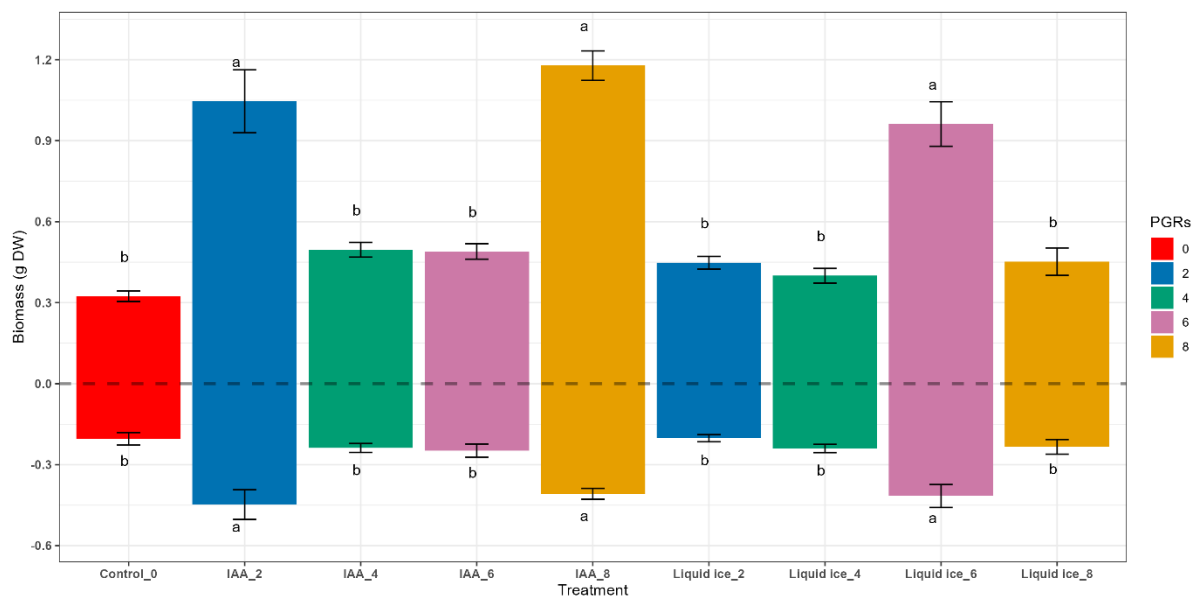


Figure 2. Biomass responses to IAA and Liquid Ice across exposure levels. Bar plot showing mean biomass (g DW) partitioned into above-ground and below-ground components for Control (0), IAA (2–8), and Liquid Ice (2–8) treatments. Values on the positive y-axis represent above-ground biomass, while values on the negative y-axis represent below-ground biomass, plotted on the same absolute scale. Error bars represent mean $\pm$ 1 SE ($n = 5$). Letters above bars denote statistical groupings from post-hoc comparisons, where treatments sharing a letter are not significantly different. The dashed horizontal line marks the zero-reference separating above and below-ground biomass.

Below-ground biomass followed a similar pattern, indicating coordinated enhancement of whole-plant growth rather than a shift in allocation solely toward harvestable shoot biomass. IAA_2, IAA_8, and LI_6 increased below-ground biomass by 120%, 100%, and 104%, respectively, relative to the control (Table 2), while LI_2 produced negligible change (-1%) despite modest gains in shoot biomass. Root:shoot ratio varied significantly ($p < 0.05$) among treatments but showed no clear evidence of major shifts in biomass partitioning, although the lowest value was observed under IAA_8 (0.35 ± 0.01 ; Table S1), reflecting proportionally greater increases in above-ground biomass. Root density scores differed significantly among treatments ($p < 0.05$) and were highest under IAA_8 (2.73 ± 0.25), followed by LI_6 and the shorter IAA exposures, consistent with the more extensive root systems observed visually in washed root samples (Figure S2). Representative canopy images also showed greater shoot elongation and canopy expansion under LI_6 and IAA_8 relative to the control and intermediate treatments (Figure S3). Tiller and bud number did not differ significantly among treatments (Table S1), indicating that treatment-driven biomass gains were primarily associated with enhanced elongation and structural expansion rather than increased tillering or bud initiation.

Pairwise correlation analysis further indicated that final height and rapid-phase growth rate were positively associated with both above- and below-ground biomass, while root density was positively associated with below-ground biomass and root:shoot ratio was negatively associated with above-ground biomass (Figure S1). Based on these responses, IAA_2, IAA_8, and LI_6 were noted as duration-optimised conditioning regimes for the subsequent priming experiment.

Post-transplant establishment and growth dynamics following conditioning and priming

All plants successfully re-established following transplanting, and no mortality was observed throughout the experimental period. Time to 50% regrowth (T_{50}) did not differ significantly among treatments ($p > 0.05$; Table 3), indicating that neither conditioning nor priming substantially altered the timing of regrowth following transplanting. Nevertheless, primed treatments exhibited numerically shorter T_{50} values than the untreated control, with LI_1 showing the lowest mean value (12.80 ± 1.80 days) compared with 16.40 ± 2.20 days in the control.

Table 32. Showing the effect of pgr conditioning and priming on selected morphological, physiological and biochemical parameter of *miscanthus* [T₅₀: time to 50 % regrowth, R:S: root-shoot ratio, M-PTE: minor & potentially toxic elements, MAE: Major ash-forming elements, HHV: high heating value, C:N: Carbon-Nitrogen ratio, LEF: linear electron flow, ΦNPQ: non-photochemical quenching, ΦII: quantum yield of photosystem II, SPAD: relative chlorophyll, AnthM: Anthocyanin, ChlM: Chlorophyll, FlvM: Flavonol, NFI: Nitrogen-Flavonol]

Parameter	CTRL	IAA_0	IAA_1	LI_0	LI_1
T ₅₀	16.40 ± 2.20 ^a	15.00 ± 2.07 ^a	14.60 ± 2.20 ^a	14.60 ± 2.20 ^a	12.80 ± 1.80 ^a
R:S	0.61 ± 0.02 ^a	0.62 ± 0.07 ^a	0.56 ± 0.02 ^a	0.71 ± 0.06 ^a	0.67 ± 0.04 ^a
Total ash (%)	3.88 ± 0.25 ^a	3.55 ± 0.47 ^a	3.25 ± 0.26 ^a	4.18 ± 0.27 ^a	3.40 ± 0.42 ^a
Total MAE (%)	99.72 ± 0.02 ^a	99.67 ± 0.02 ^{ab}	99.73 ± 0.0 ^a	99.63 ± 0.02 ^b	99.67 ± 0.00 ^{ab}
Total M-PTE (%)	0.02 ± 0.00 ^a	0.02 ± 0.00 ^a	0.03 ± 0.00 ^a	0.02 ± 0.00 ^a	0.02 ± 0.00 ^a
HHV (MJkg ⁻¹)	15.50 ± 0.53 ^b	17.80 ± 0.14 ^a	17.90 ± 0.47 ^a	18.50 ± 0.32 ^a	17.80 ± 0.26 ^a
Carbon (% DW)	46.0 ± 0.36 ^a	45.80 ± 0.30 ^a	45.80 ± 0.27 ^a	46.20 ± 0.10 ^a	45.90 ± 0.13 ^a
Nitrogen (%DW)	0.25 ± 0.09 ^a	0.12 ± 0.02 ^b	0.10 ± 0.03 ^b	0.15 ± 0.06 ^{ab}	0.12 ± 0.04 ^b
*C:N	2.89 ± 0.91 ^a	4.45 ± 0.82 ^a	4.35 ± 0.95 ^a	4.91 ± 1.26 ^a	5.37 ± 1.38 ^a
LEF	54.90 ± 9.8 ^b	108.00 ± 0.21 ^{ab}	120.00 ± 0.27 ^a	73.40 ± 0.22 ^{ab}	58.50 ± 0.14 ^b
ΦNPQ	0.28 ± 0.05 ^a	0.34 ± 0.05 ^a	0.26 ± 0.06 ^a	0.32 ± 0.08 ^a	0.28 ± 0.07 ^a
ΦII	0.52 ± 0.01 ^a	0.40 ± 0.04 ^a	0.50 ± 0.03 ^a	0.46 ± 0.01 ^a	0.48 ± 0.05 ^a
SPAD	22.00 ± 0.84 ^a	17.20 ± 2.10 ^a	18.90 ± 2.79 ^a	19.00 ± 1.11 ^a	20.00 ± 1.51 ^a
AnthM	0.12 ± 0.03 ^a	0.17 ± 0.03 ^a	0.13 ± 0.04 ^a	0.12 ± 0.01 ^a	0.13 ± 0.03 ^a
ChlM	0.31 ± 0.05 ^a	0.25 ± 0.03 ^a	0.30 ± 0.05 ^a	0.33 ± 0.02 ^a	0.34 ± 0.01 ^a
FlvM	1.28 ± 0.04 ^a	1.27 ± 0.02 ^a	1.29 ± 0.03 ^a	1.21 ± 0.07 ^a	1.25 ± 0.06 ^a
NFI	0.24 ± 0.03 ^a	0.20 ± 0.02 ^a	0.23 ± 0.04 ^a	0.28 ± 0.04 ^a	0.28 ± 0.02 ^a
Lutein (µg g ⁻¹ DW)	29.70 ± 1.95 ^a	27.80 ± 2.68 ^a	12.30 ± 2.75 ^c	20.60 ± 4.60 ^{ab}	17.30 ± 2.26 ^{bc}
β-carotene (µg g ⁻¹ DW)	21.10 ± 0.95 ^a	19.20 ± 1.84 ^a	10.10 ± 1.62 ^c	16.80 ± 2.60 ^{ab}	15.20 ± 2.27 ^{bc}

Means followed by different lowercase letters are significantly different according to Tukey's HSD test ($P < 0.05$).

*Mean value = actual value/100. N = 5.

Stretched height increased progressively over the 150-day post-transplant period across all treatments, with temporal progression remaining the dominant driver of growth (Figure 3a). During the early growth stage, treatment effects were limited and all plants exhibited broadly similar height trajectories (Figure 3b). However, during the rapid growth stage, conditioned plants separated from the control, and priming effects were product dependent. Within IAA-conditioned plants, priming increased stretched height during the rapid growth stage, with IAA_1 exceeding IAA_0. In contrast, within Liquid Ice-conditioned plants, priming reduced stretched height, with LI_1 consistently lower than LI_0. LI_0 exhibited the greatest stretched height during much of this period, followed by IAA_1, whereas control plants remained shortest throughout much of the rapid elongation phase. By the saturation/late stage, treatment differences diminished and final stretched heights converged, although conditioned plants generally remained numerically taller than controls.

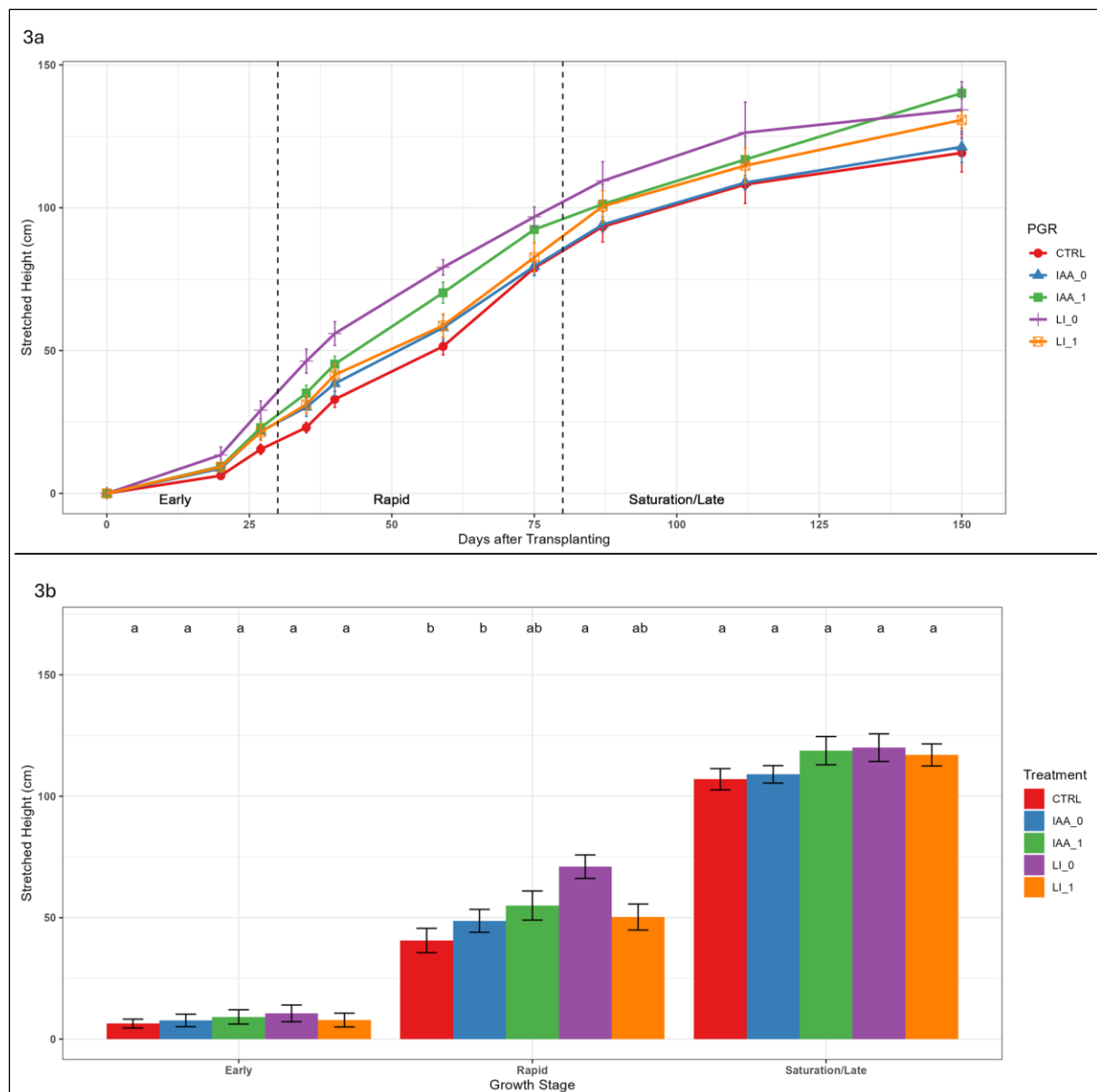


Figure 3. Effects of short-duration PGR conditioning and priming on stretched height development of seed-derived *Miscanthus* plugs following transplanting. (a) Temporal progression of stretched height (cm) measured at regular intervals up to 150 days after transplanting for control plants (CTRL), IAA-conditioned plugs without priming (IAA_0), IAA-conditioned plugs with 1-h priming (IAA_1), Liquid Ice-conditioned plugs without priming (LI_0), and Liquid Ice-conditioned plugs with 1-h priming (LI_1). Values represent means $\pm$ SE ($n = 5$). (b) Mean stretched height grouped into three growth stages—Early, Rapid, and Saturation/Late—derived from days after transplanting. Bars represent means $\pm$ SE ($n = 5$). Different letters above bars indicate significant differences among treatments within a growth stage based on Tukey's HSD test ($P < 0.05$).

Final heights were highest under IAA_1 and LI_0, reaching approximately 140 cm and 126 cm, respectively, compared with approximately 120 cm in the control (Figure 3a). Similar temporal patterns were observed for ligule height, tiller number, and leaf number, with strong seasonal progression but limited treatment-driven separation (Figures S4–S6). These results indicate that conditioning and priming primarily altered the rate of post-

transplant growth during the rapid elongation phase rather than affecting early establishment or ultimate canopy height.

Biomass allocation and biomass-quality responses following conditioning and priming

At final harvest, all conditioned and/or primed treatments produced greater total biomass than the untreated control, with particularly strong responses in above-ground biomass (Figure 4). Above-ground biomass differed significantly among treatments, with the highest accumulation observed under IAA_1 and LI_0. Within IAA-conditioned plants, priming increased above-ground biomass relative to conditioning alone, whereas within Liquid Ice-conditioned plants, priming reduced above-ground biomass relative to unprimed (LI_0). These treatments, together with IAA_0, were significantly greater than the control, whereas LI_1 was intermediate and not significantly different from either group. Below-ground biomass followed a broadly similar trend, although statistical separation was weaker. LI_0 exhibited the greatest below-ground biomass, while IAA_0 and IAA_1 were intermediate; the control consistently had the lowest below-ground biomass. As with above-ground biomass, priming under Liquid Ice reduced below-ground biomass relative to conditioning alone.

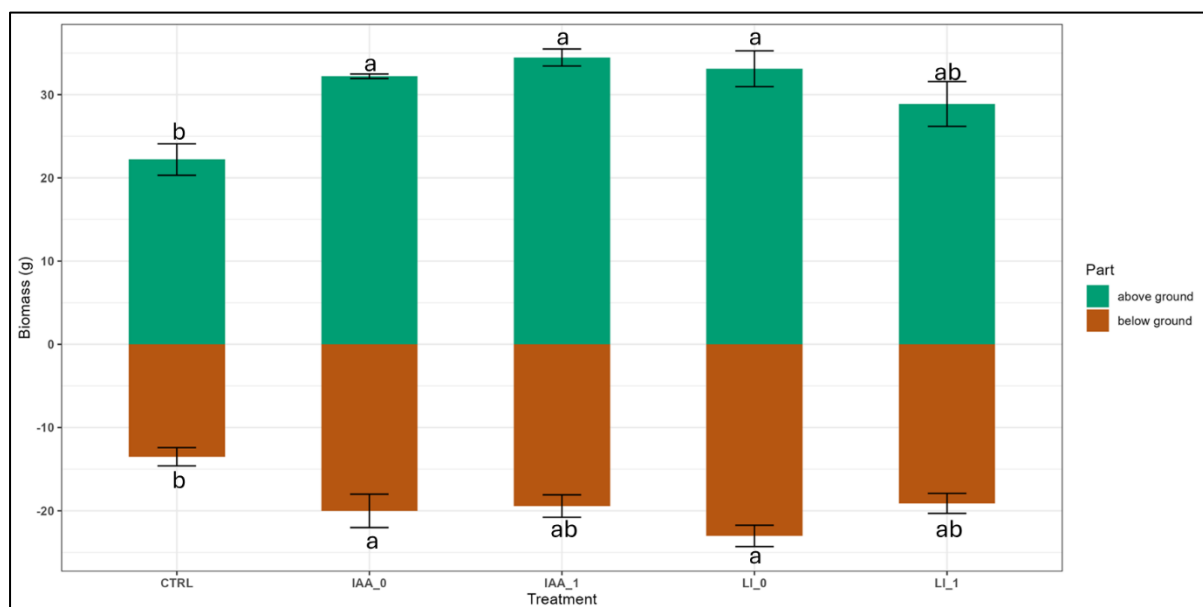


Figure 4. Partitioned biomass accumulation in seed-derived *Miscanthus* plugs following PGR conditioning and priming treatments. Mean ($\pm$ SE) above-ground (positive bars) and below-ground (negative bars) dry biomass of *Miscanthus* plants subjected to control (CTRL), auxin conditioning without priming (IAA_0), auxin conditioning with priming (IAA_1), liquid ice conditioning without priming (LI_0), and liquid ice conditioning with priming (LI_1). Bars represent treatment means; error bars denote standard error; $n = 5$. Different lowercase letters above or below bars

indicate significant differences among treatments within each biomass compartment based on ANOVA followed by Tukey's HSD test ($\alpha = 0.05$).

Despite these increases in both biomass compartments, root:shoot ratio did not differ significantly among treatments ($P > 0.05$; Table 3), indicating that treatment-driven gains were broadly proportional across above- and below-ground tissues rather than reflecting altered allocation patterns. Biomass quality traits were largely maintained or improved following conditioning and priming (Table 3). Total ash content did not differ significantly among treatments ($p > 0.05$), although LI_0 exhibited numerically higher values than the control. In contrast, total major ash-forming elements (MAE) differed significantly ($p < 0.05$), with LI_0 exhibiting the lowest MAE concentration, while minor and potentially toxic elements (M-PTE) were unaffected; detailed elemental composition is presented in Figure S7. Higher heating value (HHV) differed strongly among treatments ($p < 0.05$), with all conditioned and/or primed plants exhibiting significantly greater HHV than the control. LI_0 produced the highest HHV ($18.50 \pm 0.32 \text{ MJ kg}^{-1}$), followed closely by IAA_1 and the remaining treated groups, whereas the control exhibited the lowest value ($15.50 \pm 0.53 \text{ MJ kg}^{-1}$). Carbon concentration remained unchanged across treatments ($p > 0.05$), while nitrogen concentration differed significantly ($p < 0.05$), with lower values in most treated plants relative to the control. However, C:N ratio did not differ significantly. These results show that conditioning and priming increased biomass accumulation and energy density without increasing ash concentration or causing major deterioration in biomass-quality characteristics.

Physiological and biochemical responses to conditioning and priming

Conditioning and priming significantly altered selected physiological traits, particularly those associated with photosynthetic electron transport (Table 3). Linear electron flow (LEF) differed significantly among treatments ($p < 0.05$), with the highest values observed under IAA_1, followed by IAA_0 and LI_0. Within IAA-conditioned plants, priming increased LEF relative to conditioning alone, whereas within Liquid Ice-conditioned plants, priming reduced LEF relative to LI_0. Control and LI_1 exhibited the lowest LEF values. In contrast, neither non-photochemical quenching (Φ_{NPQ}) nor quantum yield of photosystem II (Φ_{II}) differed significantly among treatments, indicating that increases in electron transport occurred without detectable changes in regulated thermal dissipation

or PSII operating efficiency. Similarly, relative chlorophyll content (SPAD) did not differ significantly among treatments, suggesting that the observed increases in LEF were not associated with major changes in total chlorophyll concentration.

Pigment meter indices, including anthocyanin (AnthM), chlorophyll (ChlM), flavonol (FlvM), and nitrogen-flavonol index (NFI), did not differ significantly among treatments ($p > 0.05$; Table 3), indicating limited treatment effects on general leaf pigment status as assessed by non-destructive optical measurements. In contrast, carotenoid concentrations were significantly affected ($p < 0.05$). Trans-lutein differed significantly among treatments ($p < 0.05$), with the highest concentrations observed in the control and IAA_0 and the lowest in IAA_1. Similarly, within IAA-conditioned plants, priming reduced both trans-lutein and trans- β -carotene relative to IAA_0. Liquid Ice treatments were intermediate but generally lower than the control. A similar pattern was observed for trans- β -carotene ($p < 0.05$), with IAA_1 again exhibiting the lowest concentration. Representative chromatograms and calibration curves used for carotenoid identification and quantification are shown in Figure S9. Overall, the most pronounced physiological and biochemical responses were observed under IAA_1, whereas priming under Liquid Ice did not confer comparable physiological advantages.

DISCUSSION

Growth responses to growth-regulating compounds are non-linear and product-specific during plug development

The present study demonstrates that the growth responses of seed-derived *Miscanthus* plugs to growth-regulating compounds (GRC) are strongly dependent on both product type and cumulative exposure duration during nursery-stage development. Across the conditioning experiment, both the plant growth regulator (indole-3-acetic acid ; IAA) and the biostimulant (Liquid Ice; LI) product produced distinctly non-linear response patterns, indicating that increasing exposure duration did not result in a progressive or proportional increase in growth across products. Instead, optimal responses occurred at distinct exposure windows. IAA exhibited a bimodal response, with growth optima occurring under both short (IAA_2) and prolonged (IAA_8) exposure durations, whereas Liquid Ice exhibited a unimodal response, with peak performance under intermediate

exposure (IAA_6) followed by reduced growth under prolonged application. Such non-linear responses are consistent with the established principle that plant responses to exogenous growth regulators depend on interactions among concentration, exposure duration, developmental stage, and endogenous physiological status rather than cumulative exposure alone (Teale *et al.*, 2006; Pacurar *et al.*, 2014; Kurepa *et al.*, 2018).

The non-linear responses observed under IAA are consistent with the complexity of auxin-mediated developmental regulation. Auxins regulate root initiation, vascular differentiation, cell elongation, apical dominance, and source–sink relationships through tightly coordinated signalling pathways and interactions with cytokinin, gibberellin, and ethylene (Woodward & Bartel, 2005; Davies, 2010). In horticultural and forestry nursery systems, exogenous auxin treatments are commonly used to improve rooting and seedling quality, but their effects are strongly dependent on concentration, timing, and duration of exposure (Teale *et al.*, 2006; Pacurar *et al.*, 2014). Low or transient exposure can stimulate root initiation and vegetative expansion, whereas prolonged or excessive exposure may suppress leaf expansion, alter assimilate partitioning, or trigger feedback inhibition through altered hormonal homeostasis (Keller *et al.*, 2004; Kurepa *et al.*, 2018). The strong performance of IAA_2 in the present study may indicate that brief early exposure was sufficient to trigger developmental or physiological priming effects that persisted after treatment withdrawal, allowing enhanced growth to continue during subsequent nursery development. In contrast, the strong response under IAA_8 suggests that continued auxin exposure remained stimulatory under later nursery-stage conditions and supported ongoing elongation and structural development. The weaker responses observed at intermediate durations may reflect a transient overlap between stimulatory and inhibitory phases of auxin signalling, whereby early developmental stimulation was offset by later feedback inhibition or altered source–sink regulation before plants transitioned into a more elongation-responsive developmental stage. Similar biphasic or threshold-dependent auxin responses have been reported in other crops and model species, where developmental timing influences whether exogenous auxin is promotive or inhibitory (Kurepa *et al.*, 2018; Zhang *et al.*, 2022; Li and Chen, 2023).

The response to Liquid Ice likely reflects the more complex and multi-component nature of BS-mediated regulation. Unlike auxins, BS formulations typically act through multiple overlapping pathways, including improved nutrient assimilation, osmotic adjustment, antioxidant metabolism, and modulation of endogenous hormonal signalling (du Jardin, 2015; Rouphael & Colla, 2020). In nursery-stage horticultural systems, BS applications have been reported to improve plug quality and transplant readiness through enhanced root growth, nutrient-use efficiency, and stress resilience (Rouphael & Colla, 2020). However, responses are often inconsistent and product-specific, particularly when application frequency or dose exceeds optimal thresholds (Calvo *et al.*, 2014; Yakhin *et al.*, 2017). In perennial energy crops, comparable variability has been observed. For example, Pidlisnyuk *et al.* (2022) reported improved growth and physiological performance in *Miscanthus × giganteus* under some stimulant treatments, whereas Katelevskij *et al.* (2020) found treatment effects to be variable depending on formulation and environmental conditions. The strong performance of LI_6 suggests that intermediate-duration exposure was sufficient to induce beneficial physiological changes, whereas prolonged exposure under LI_8 may have exceeded the optimum threshold or imposed metabolic costs that reduced subsequent elongation and biomass accumulation.

A notable observation was that treatment effects were most pronounced during the rapid growth phase rather than during early establishment or late-stage growth. This indicates that GRC exposure during nursery development did not necessarily produce immediate visible gains but altered developmental momentum that became expressed during the elongation phase. The positive correlations observed between rapid-phase growth rate, final height, and both above- and below-ground biomass (Figure S1) support this interpretation. In grasses, rapid canopy expansion during early ontogeny strongly influences cumulative light interception and carbon assimilation (Poorter *et al.*, 2012, Robson *et al.*, 2013; 2025). In *Miscanthus*, a large proportion of annual biomass accumulation occurs during a relatively short period of rapid stem elongation and canopy expansion (Hastings *et al.*, 2017). In the context of plug production, treatments that accelerate growth during this phase may improve plug quality at transplanting and influence subsequent post-transplant performance.

Biomass accumulation and below-ground development are critical outcomes for plug quality and establishment potential

Enhanced biomass accumulation was one of the most consistent outcomes of GRC treatment during nursery-stage plug development. Treatments that increased rapid growth phase elongation also produced greater above-ground biomass at harvest, indicating that stimulation during key developmental windows translated into larger and potentially more vigorous plugs at transplanting. In plug-based production systems, greater above-ground biomass can increase photosynthetic capacity and improve resilience during the transition from controlled nursery conditions to more variable post-transplant environments. In horticultural transplant systems, increases in canopy size and structural biomass during nursery production are often associated with improved transplant quality and faster establishment after transplanting (Colla *et al.*, 2014; Bulgari *et al.*, 2019).

The increases in below-ground biomass observed under IAA_2, IAA_8, and LI_6 are particularly important because root system quality is a major determinant of transplant success. Root biomass and architecture influence anchorage, nutrient and water acquisition, and the ability to withstand transplant shock. In perennial species such as *Miscanthus*, improved root and rhizome development at transplanting may also support overwinter survival and subsequent-year regrowth once established in the field (Atkinson, 2009; Clifton-Brown *et al.*, 2017). Ashman *et al.* (2023) showed that plug tray design influenced both above- and below-ground development in seed-derived *Miscanthus*, reinforcing the importance of nursery-stage plug quality in determining establishment potential. The improved root density and visual root architecture observed here (Figure S2) therefore suggest that the strongest-performing treatments may improve both transplant quality and later field establishment.

Published studies directly evaluating GRC applications during nursery-stage *Miscanthus* plug development are currently lacking. However, the post-transplant responses observed here are broadly consistent with previous studies in established or rhizome-derived *Miscanthus* plants. For example, Pidlisnyuk *et al.* (2022) reported increased height and biomass in response to selected growth stimulants under field conditions, although responses were treatment- and environment-dependent. Conversely,

Katelevskij *et al.* (2020) reported more variable responses, highlighting the importance of product chemistry and environmental context. The present study contributes to this literature by showing that manipulating developmental trajectories during nursery-stage plug production may influence later performance before field establishment.

Despite increases in both above- and below-ground biomass, root:shoot ratio remained stable across treatments, indicating coordinated whole-plant growth rather than altered biomass partitioning. This is consistent with broader ecological analyses showing that increases in productivity frequently occur without major changes in allocation ratios due to ontogenetic and allometric constraints (Poorter *et al.*, 2012). From a practical perspective, such proportional gains are desirable because they improve plug vigour without compromising below-ground investment needed for persistence after transplanting.

Priming responses in this study depended on pre-conditioning history

A central finding of the post-transplant experiment was that the effectiveness of priming depended strongly on prior conditioning history. Within IAA-conditioned plants, priming improved stretched height during the rapid growth phase, increased above-ground biomass, and increased linear electron flow (LEF) relative to conditioning alone. In contrast, within Liquid Ice-conditioned plants, priming reduced stretched height, biomass accumulation, and LEF relative to LI_0. These contrasting responses indicate that priming efficacy can vary depending on the physiological state established during prior conditioning and that the same intervention may be beneficial, neutral, or inhibitory depending on pre-existing plant status.

Published studies quantifying priming responses in seed-derived *Miscanthus* plugs are currently lacking, making direct comparison within this crop limited. However, the beneficial effects of IAA priming observed here are broadly consistent with findings in transplanted horticultural systems where hormone-based or BS-based pre-transplant treatments improve post-transplant performance (Van Oosten *et al.*, 2017; Rouphael & Colla, 2020). Auxin-based dips and root-zone treatments are widely used to stimulate rooting and reduce transplant shock in vegetable, ornamental, and forestry systems (Davies, 2010). The increased LEF observed under IAA_1 may indicate enhanced

photosynthetic activation and faster restoration of carbon assimilation following transplanting, thereby supporting faster canopy expansion and biomass accumulation.

In contrast, the reduced or neutral effects of priming under Liquid Ice differ from some reports of positive BS priming effects in crops such as maize, wheat, and tomato, where enhanced nutrient uptake, antioxidant metabolism, and stress resilience have been observed (Calvo *et al.*, 2014; Rouphael & Colla, 2020). However, inconsistent or threshold-dependent responses to BS are also widely documented, particularly where products are repeatedly applied or where plants are not under strong abiotic stress (Yakhin *et al.*, 2017). It is plausible that LI_0 (LI_6 in nursery-phase) had already induced a near-optimal physiological state and that additional exposure provided limited benefit or imposed metabolic or signalling costs. Similar diminishing-return responses have been reported in repeated biostimulant applications in other crops (Yakhin *et al.*, 2017). From a commercial perspective, these findings suggest that priming should not be assumed universally beneficial and may need to be tailored according to prior conditioning history and product chemistry.

Biomass quality improved without major trade-offs

An important consideration when increasing early biomass productivity is whether gains are accompanied by changes in biomass quality. In the present study, increased biomass accumulation following conditioning and priming was not associated with deterioration in the measured fuel-quality traits at the time of assessment. Total ash content remained stable across treatments despite substantial gains in biomass, indicating that productivity enhancement did not increase ash-related penalties for thermochemical conversion. This is particularly relevant because ash content and inorganic composition strongly influence slagging, fouling, corrosion, and overall conversion efficiency in combustion and other thermochemical systems (Jenkins *et al.*, 1998; Baxter *et al.*, 2012; van der Weijde *et al.*, 2017).

Although relatively few studies have examined the effects of growth-regulating compounds on biomass quality traits in *Miscanthus*, previous work in established plants has shown that growth stimulants and agronomic interventions can alter biomass composition. Pidlisnyuk *et al.* (2022) reported changes in biomass composition following

stimulant application under contaminated conditions, while other studies have shown that delayed harvest or genotype selection can improve ash composition and combustion quality, often with associated trade-offs in biomass yield (Iqbal *et al.*, 2017; Endriss *et al.*, 2024). In contrast, the present treatments maintained or improved both yield and ash-related quality metrics, suggesting that selected GRC interventions may improve both quantity and quality simultaneously.

The reduced concentration of major ash-forming elements under LI_0 further supports improved combustion suitability, as lower concentrations of K, Cl, and S are associated with reduced ash melting, corrosion, and particulate emissions (van der Weijde *et al.*, 2017). Higher heating value (HHV) was also significantly greater in all treated plants than in the control, indicating improved biomass energy density. Similar variation in HHV has been reported in *Miscanthus* under differing management practices and harvest timings, demonstrating that energy density is not fixed and can respond to compositional or physiological shifts (Greenhalf *et al.*, 2013; Nebeská *et al.*, 2019). Although the increases observed here were modest in absolute terms, when combined with increased biomass production they represent meaningful gains in total energy yield per plant. Nitrogen concentration was generally lower in treated plants, while C:N ratio remained unchanged, suggesting that energy gains were not offset by increased nitrogen-related emissions risk.

However, these compositional measurements were obtained from young plants before overwintering and substantially before the stage at which *Miscanthus* biomass would normally be harvested commercially. Consequently, the observed stability or improvement in biomass-quality traits represents an early-stage response and cannot be assumed to persist to the first commercial harvest. Biomass composition can change with crop age, environmental conditions and harvest timing, with established *Miscanthus* generally showing improved combustion quality following later or delayed harvest (Clifton-brown & Lewandowski 2002; Iqbal *et al.*, 2017). Longer-term assessment would therefore be required to determine whether the apparent absence of quality trade-offs following conditioning and priming is maintained at harvest.

Physiological and biochemical responses provide insight into treatment effects

The physiological responses observed following conditioning and priming provide insight into possible mechanisms underpinning treatment effects. LEF was highest under IAA_1 and generally greater under the strongest-performing treatments, suggesting increased photosynthetic electron transport capacity. LEF is commonly used as a proxy for light-driven electron transport and may reflect enhanced photosynthetic performance where electrons are effectively coupled to carbon assimilation or other productive sinks (Kuhlgert *et al.*, 2016; Shimakawa *et al.*, 2018). Increased fluorescence-derived electron transport has been associated with enhanced productivity in grasses and crop plants under favourable conditions (Zavřel *et al.*, 2018), supporting the interpretation that higher LEF may have contributed to the greater biomass accumulation observed here.

The absence of treatment effects on Φ_{NPQ} and Φ_{II} indicates that increased LEF was not associated with increased regulated energy dissipation or altered PSII operating efficiency. Similar patterns have been interpreted as evidence of improved photochemical utilisation rather than increased excitation pressure (Kramer *et al.*, 2004; Klughammer & Schreiber, 2008). Likewise, the absence of treatment effects on SPAD and other optical pigment indices suggests that the observed physiological changes were not associated with major shifts in general leaf pigment status. Together, these results suggest that improved photosynthetic efficiency may have occurred through enhanced electron transport utilisation rather than increased chlorophyll investment or altered photoprotective demand.

In contrast, carotenoid concentrations declined under LI treatments and particularly under IAA_1. Carotenoids such as lutein and β -carotene play important roles in photoprotection and oxidative stress mitigation through energy dissipation and reactive oxygen scavenging (Demmig-Adams & Adams, 2002; Ruban, 2016). Lower carotenoid concentrations in the strongest-performing treatments may reflect reduced photoprotective demand under more balanced photosynthetic conditions or altered allocation of metabolic resources. Similar reductions in carotenoid investment under improved photochemical utilisation have been reported in plants experiencing lower excitation pressure or more efficient energy use (Johnson *et al.*, 2007). However, because

carotenoid metabolism is dynamic and influenced by developmental and environmental factors, this interpretation should remain cautious.

Practical implications and study limitations

From a practical perspective, the findings of this study suggest that short-duration GRC interventions may provide a scalable route to improving seed-derived *Miscanthus* plug production. Conditioning treatments of limited duration produced measurable and, in some cases, substantial gains in growth, biomass accumulation, and energy density without requiring continuous application. Furthermore, the contrasting responses to priming indicate that intervention strategies may need to be tailored to product type: IAA priming may be beneficial following conditioning, whereas Liquid Ice priming may be unnecessary or counterproductive. Such optimisation is highly relevant to commercial systems where treatment cost, labour requirements, and greenhouse residence time directly affect profitability and scalability.

Several limitations should be acknowledged. The experiments were conducted under controlled glasshouse conditions and may not fully represent field environments where water limitation, temperature fluctuations, and biotic stresses influence establishment and growth. Responses may also differ across genotypes, and only one hybrid background was evaluated. Finally, endogenous hormone profiling was not performed; therefore, interpretations regarding hormonal regulation are inferred from phenotypic and physiological responses rather than direct quantification of hormonal dynamics.

Despite these limitations, the present study provides strong evidence that carefully timed conditioning and priming interventions can improve both growth and biomass quality in seed-derived *Miscanthus*. Future work should assess the persistence of these responses under field conditions, across multiple genotypes, and over multiple growing seasons to determine whether early-stage gains translate into improved long-term establishment and commercial productivity.

CONCLUSION

This study demonstrates that growth-regulating compound application during plug production can substantially influence both early development and subsequent post-

transplant performance in seed-derived *Miscanthus*. Responses were strongly non-linear and product-specific, with distinct exposure-duration optima and clear interactions between conditioning and priming. Importantly, biomass gains were achieved without compromising key biomass-quality traits, indicating that targeted interventions can improve both yield and energy value. The contrasting responses to priming in this study indicate that post-transplant treatment efficacy is influenced by prior physiological conditioning. Collectively, these findings provide a mechanistic and practical basis for optimising plug production systems and support the integration of short-duration, targeted GRC strategies to enhance the scalability and reliability of seed-derived *Miscanthus* establishment.

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Supplementary Materials

Table S1. Root:shoot ratio (R:S), tiller number, bud number, and root density (RD) under different IAA and Liquid Ice exposure treatments.

PGR	Exp	Treatment	R:S	Tiller	Bud	RD
Control	0	Control_0	0.63±0.08 ^a	1.80±0.37 ^a	1.80±0.37 ^a	1.73±0.32 ^{abc}
IAA	2	IAA_2	0.44±0.05 ^{bc}	1.20±0.20 ^a	3.00±0.32 ^a	2.47±0.27 ^{ab}
	4	IAA_4	0.48±0.03 ^{abc}	1.40±0.25 ^a	2.40±0.40 ^a	1.80±0.20 ^{abc}
	6	IAA_6	0.51±0.04 ^{abc}	1.40±0.25 ^a	2.20±0.20 ^a	2.47±0.39 ^{ab}
	8	IAA_8	0.35±0.01 ^c	1.20±0.20 ^a	3.00±0.45 ^a	2.73±0.25 ^a
Liquid ice	2	Liquid ice_2	0.46±0.03 ^{abc}	1.20±0.20 ^a	2.60±0.25 ^a	1.33±0.00 ^c
	4	Liquid ice_4	0.60±0.02 ^{ab}	1.00±0.00 ^a	2.40±0.25 ^a	1.53±0.20 ^{bc}
	6	Liquid ice_6	0.44±0.03 ^{bc}	1.00±0.00 ^a	3.00±0.45 ^a	2.60±0.19 ^{ab}
	8	Liquid ice_8	0.52±0.04 ^{abc}	1.60±0.89 ^a	2.00±0.55 ^a	1.40±0.25 ^c

Values represent mean ± SE (n = 5). Letters following means indicate statistical groupings from post-hoc comparisons, where treatments sharing a letter are not significantly different.

Table S2. Summary of factorial ANOVA p values for treatment, time, and their interaction (where applicable) across all measured stage and traits. Repeated-measures variables were analysed using linear mixed-effects models, while single-time-point variables were analysed using one-way ANOVA. Time and interaction terms are reported only for repeated-measures variables. Asterisks (*) indicate statistically significant effects at $\alpha = 0.05$. Abbreviations: Rep, establishment-phase growth rate; Rrp, rapid-phase growth rate; Rlp, late-phase growth rate; BM_A, above-ground biomass; BM_B, below-ground biomass; R:S, root-to-shoot ratio; RD, root density; MAE, major ash elements; M-PTE, minor and potentially toxic elements; HHV, higher heating value; LEF, linear electron flow; Φ II, effective quantum yield of PSII; Φ NPQ, non-photochemical quenching; SPAD, relative chlorophyll content; AnthM, anthocyanin index; ChlM, chlorophyll index; FlvM, flavonol index; NFI, nitrogen-flavonol index.

Stage	Trait	Treatment	Time	Treatment: Time
Nursery	Stretched height	0.955	<0.001*	<0.001*
	R _{ep}	0.076		
	R _{rp}	<0.001*		
	R _{lp}	0.656		
	BM _A	<0.001*		
	BM _B	<0.001*		
	R:S	<0.001*		
	Tiller	0.681		
	Bud	0.057		
	RD	<0.001*		
Transplant	Stretched height	0.394	<0.001*	0.388
	Early growth stage	0.888		
	Rapid growth stage	<0.001*		
	Late growth stage	0.062		
	Ligule height	0.205	<0.001*	0.201
	Tiller number	0.979	<0.001*	0.226
	Leaf number	0.784	<0.001*	0.456
	BM _A	0.004*		
	BM _B	0.003*		
	T ₅₀	0.762		
	R:S	0.251		
	Total ash	0.352		
	Total MAE	0.019*		
	Total M-PTE	0.294		
	HHV	<0.001*		
	Carbon	0.940		
	Nitrogen	0.002*		
	C:N	0.248		
	LEF	0.014*		
	Φ NPQ	1.000		
	Φ II	1.000		
	SPAD	0.999		
	AnthM	0.837		
	ChlM	0.504		
	FlvM	0.527		
	NFI	0.485		
	Lutein	0.002*		
	β -carotene	0.002*		

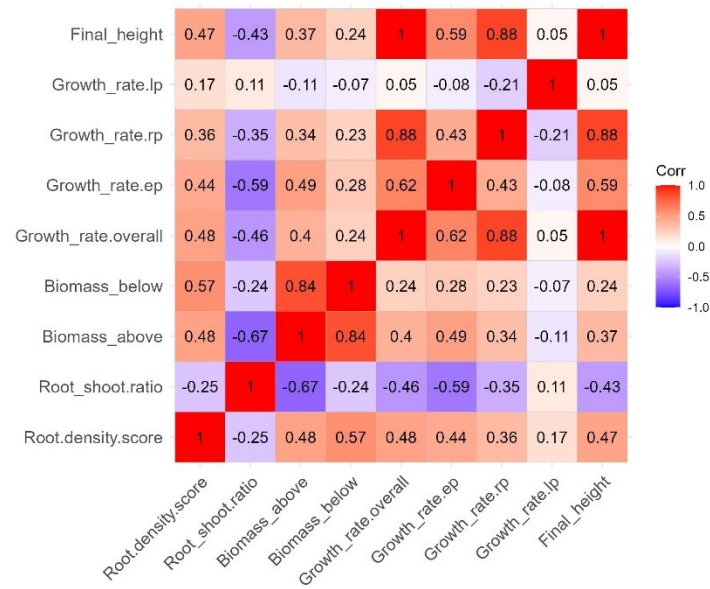


Figure S1. Correlation matrix among growth dynamics and biomass traits. Correlation matrix illustrating pairwise spearman correlation coefficients among nine growth and biomass variables: final height, growth rates (growth_rate) across early/rapid/late phases (ep/rp/lp) and overall, below-ground biomass, above-ground biomass, root:shoot ratio, and root density score. Positive correlations are shown in red and negative correlations in blue, with intensity proportional to correlation strength.

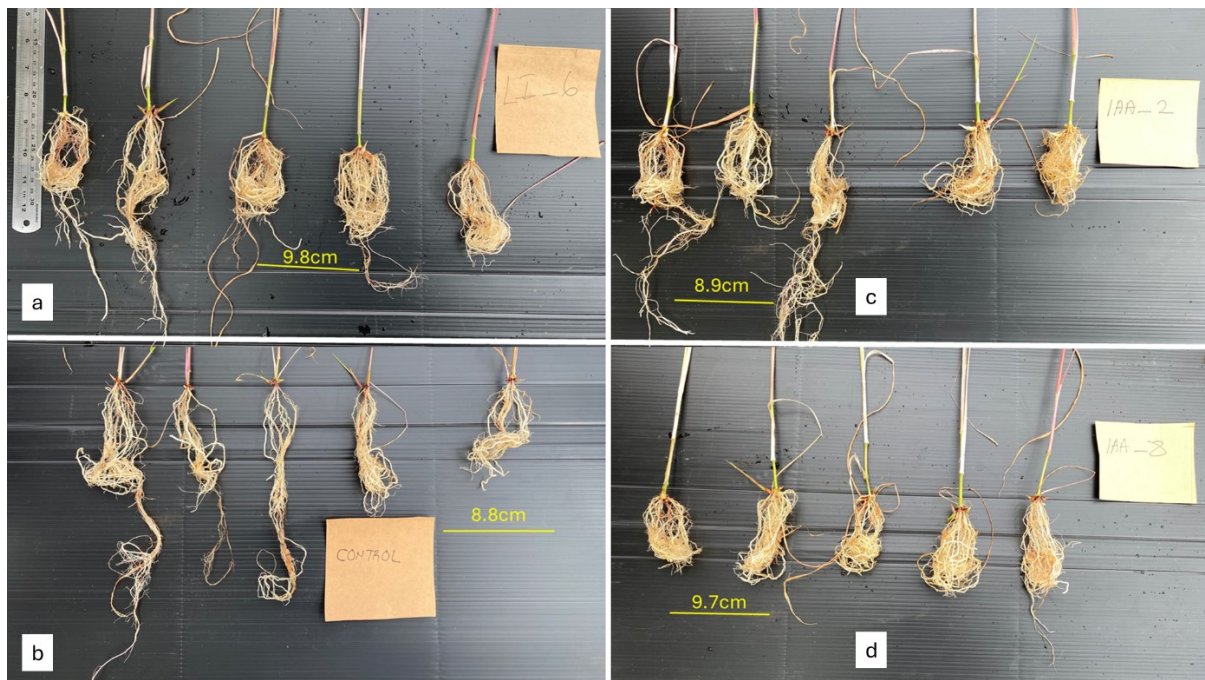


Figure S2. Representative root systems of *Miscanthus* plugs under selected PGR and biostimulant exposure treatments. Root systems of *Miscanthus* plug plants at final harvest under representative treatments illustrating differences in root architecture associated with exposure intensity. Panels show (a) Liquid Ice applied for 6 weeks (LI_6), (b) untreated control (CTRL), (c) indole-3-acetic acid applied for 2 weeks (IAA_2), and (d) indole-3-acetic acid applied for 8 weeks (IAA_8). Each panel displays five representative plants arranged after washing to remove substrate. The yellow line indicates a known reference distance. These images provide qualitative visual context for the root density and below-ground biomass measurements reported in the Results.

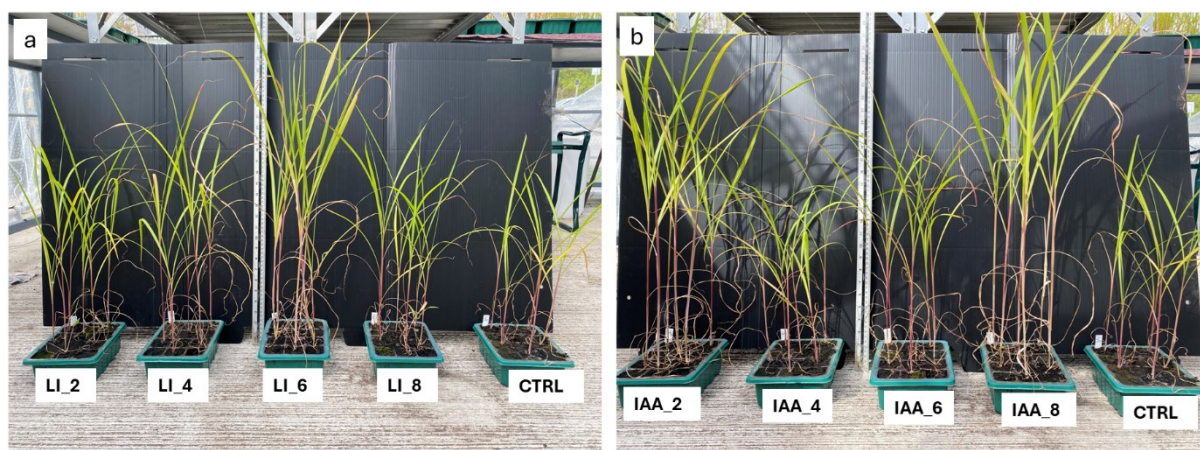


Figure S3. Representative shoot architecture of *Miscanthus* plugs under PGR and biostimulant exposure treatments. Representative shoot morphology of *Miscanthus* plug plants grown under different exposure durations of (a) Liquid Ice and (b) indole-3-acetic acid. Plants shown correspond to treatments receiving 2, 4, 6, and 8 weeks of application alongside untreated controls. Treatments associated with higher rapid growth phase elongation (notably LI_6 and IAA_8) exhibited greater canopy height and leaf expansion compared with control and intermediate treatments. These images provide visual context for the growth trajectories and elongation dynamics quantified in the main Results.

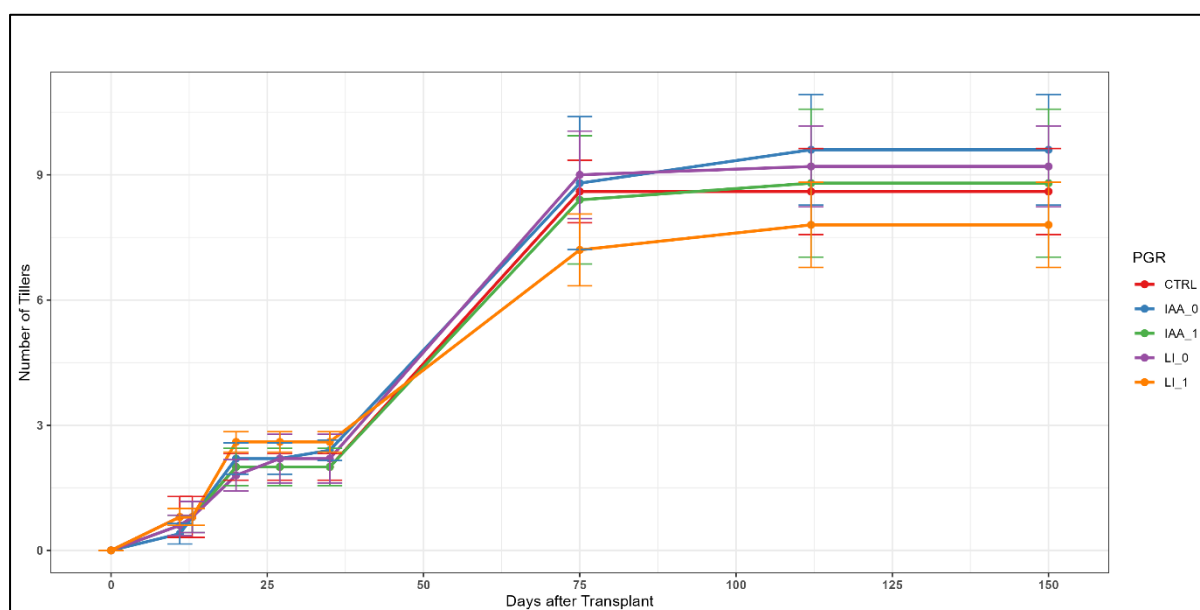


Figure S4. Temporal dynamics of tiller number in seed-derived *Miscanthus* plants following transplanting under different plant growth regulator (PGR) treatments. Mean tiller number ($\pm$ SE, $n = 5$) is shown for control plants (CTRL), IAA-conditioned plants without priming (IAA_0), IAA-conditioned plants with priming (IAA_1), LI-conditioned plants without priming (LI_0), and LI-conditioned plants with priming (LI_1) over the course of 150 days after transplanting. Data illustrate early tiller initiation, followed by a rapid increase during mid-season growth and subsequent stabilisation towards later developmental stages.

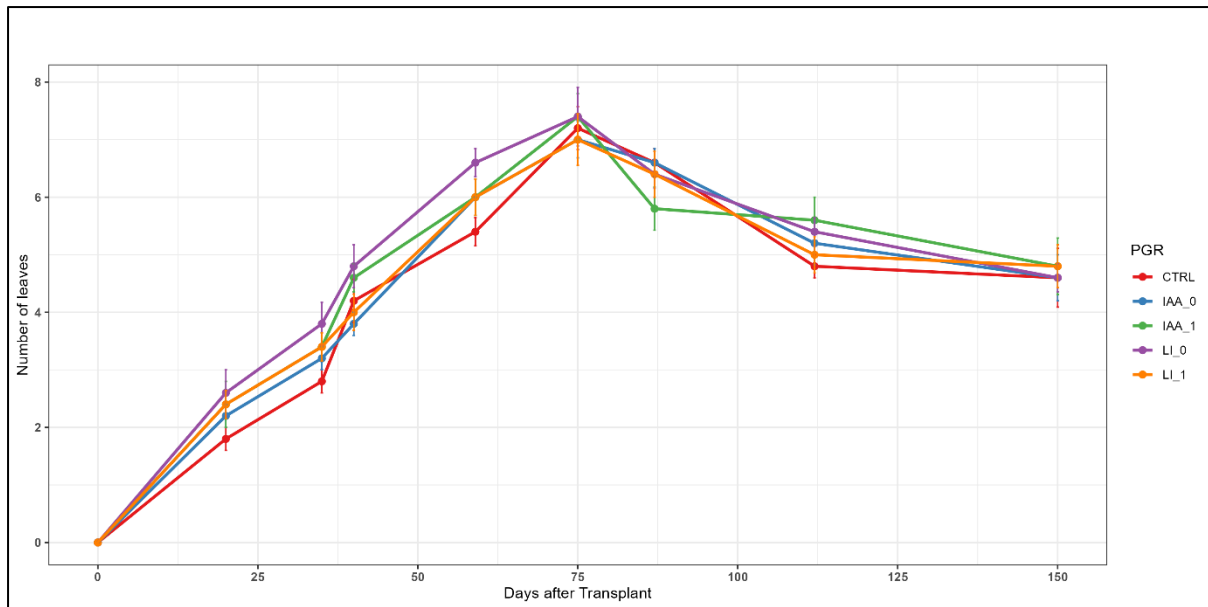


Figure S5. Changes in leaf number over time in seed-derived *Miscanthus* plants subjected to different PGR conditioning and priming treatments. Values represent mean leaf number per plant ($\pm$ SE, $n = 5$) for CTRL, IAA_0, IAA_1, LI_0, and LI_1 treatments across the experimental period. Leaf number increased during early and mid-season growth, peaking during the rapid growth phase before declining slightly towards the late season, consistent with leaf senescence and canopy turnover.

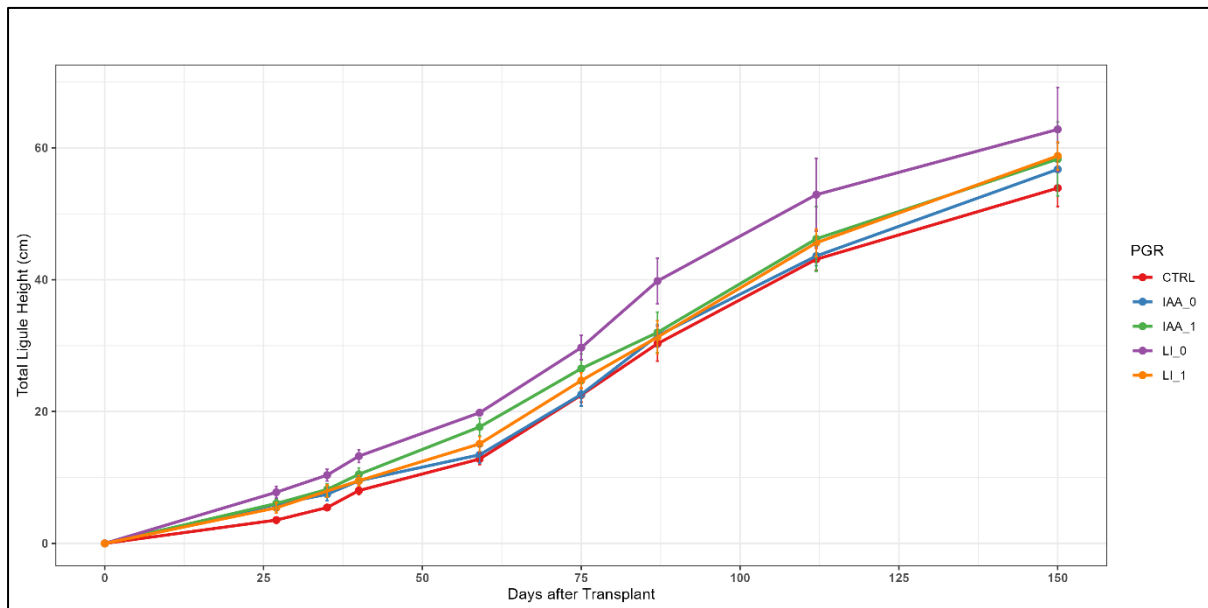


Figure S6. Temporal progression of total ligule height in *Miscanthus* plants under different PGR treatments following transplanting. Mean ligule height ($\pm$ SE, $n = 5$) is shown for CTRL, IAA_0, IAA_1, LI_0, and LI_1 treatments over 150 days after transplanting. Ligule height increased steadily throughout the growing season, reflecting cumulative stem elongation, with treatment effects generally consistent with patterns observed for stretched plant height.

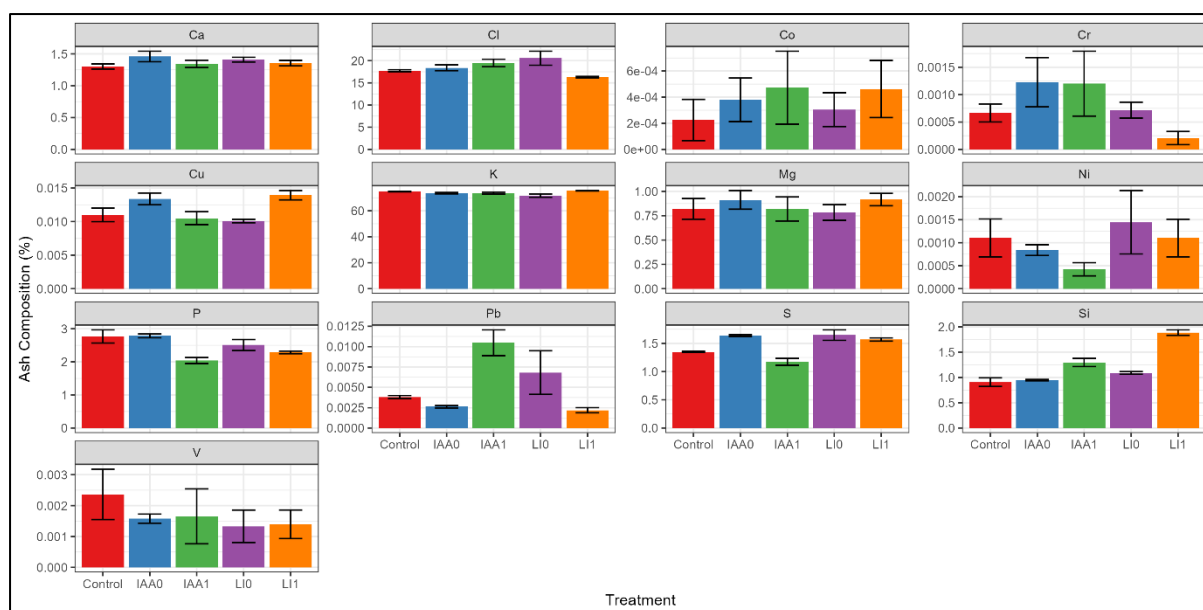


Figure S7. Elemental composition of stem ash determined by X-ray fluorescence (XRF) following furnace ashing of *Miscanthus* biomass. Bars represent mean elemental concentrations ($\pm$ SE, $n = 5$) for calcium (Ca), chlorine (Cl), cobalt (Co), chromium (Cr), copper (Cu), potassium (K), magnesium (Mg), nickel (Ni), phosphorus (P), lead (Pb), sulphur (S), silicon (Si), and vanadium (V) across five treatments: control (CTRL), IAA-conditioned without priming (IAA_0), IAA-conditioned with priming (IAA_1), LI-conditioned without priming (LI_0), and LI-conditioned with priming (LI_1). Elemental concentrations are expressed as percentage of ash (%). This figure provides a detailed breakdown of ash composition supporting grouped elemental analyses presented in the main text.

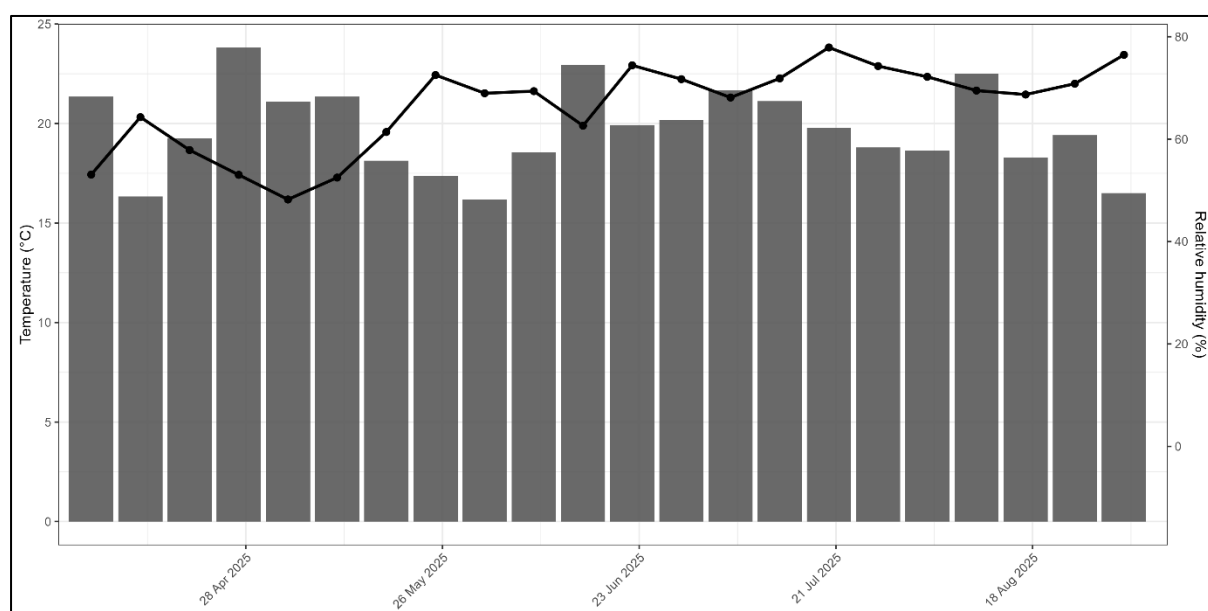


Figure S8. Weekly mean glasshouse environmental conditions during the experimental period (April–September 2025). Bars represent mean weekly air temperature (°C), averaged across two independent data loggers, while the solid line with points shows mean weekly relative humidity (%). Values were calculated from hourly measurements recorded throughout the experiment and summarised as weekly means to characterise the prevailing growth environment experienced by the plants.

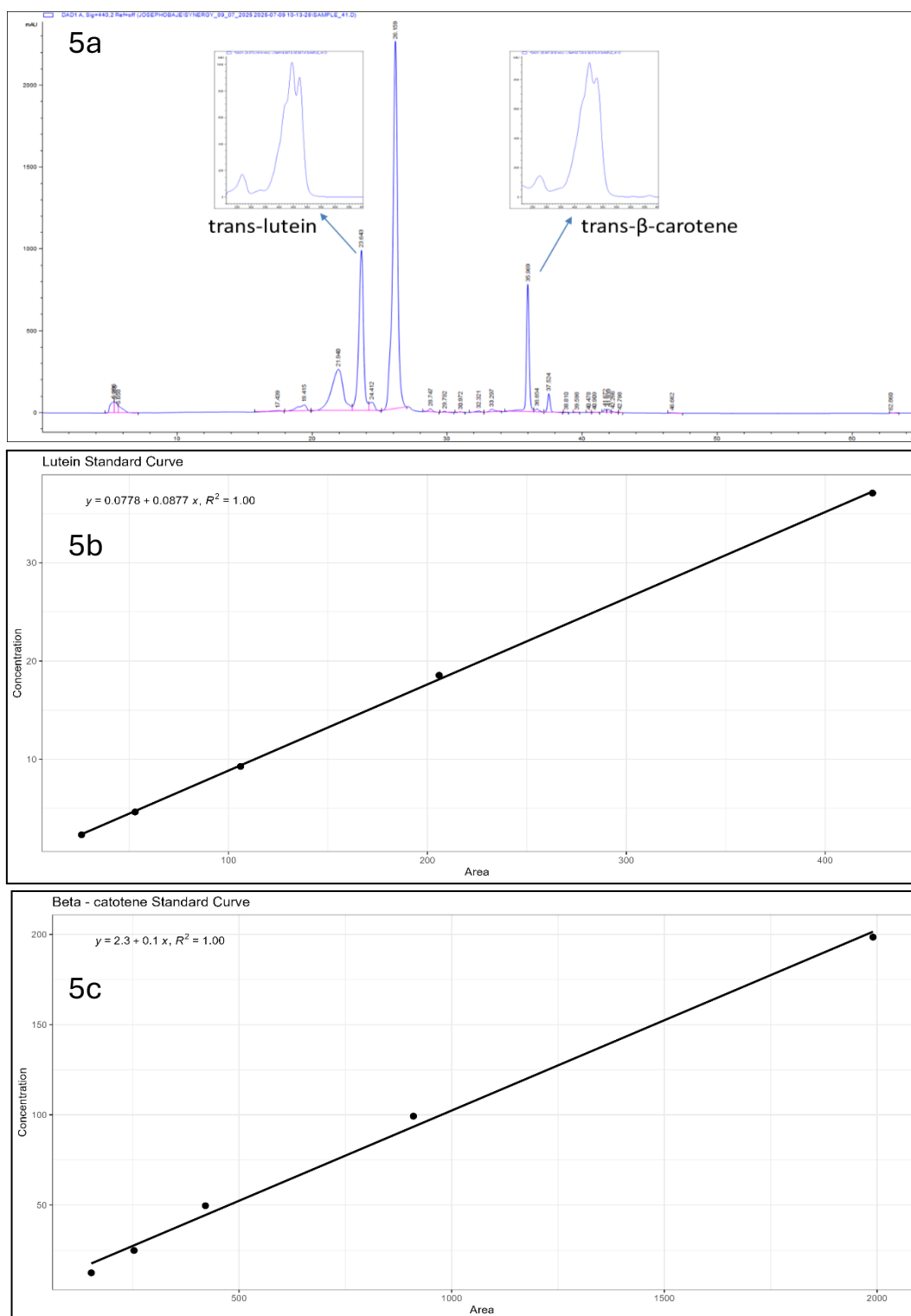
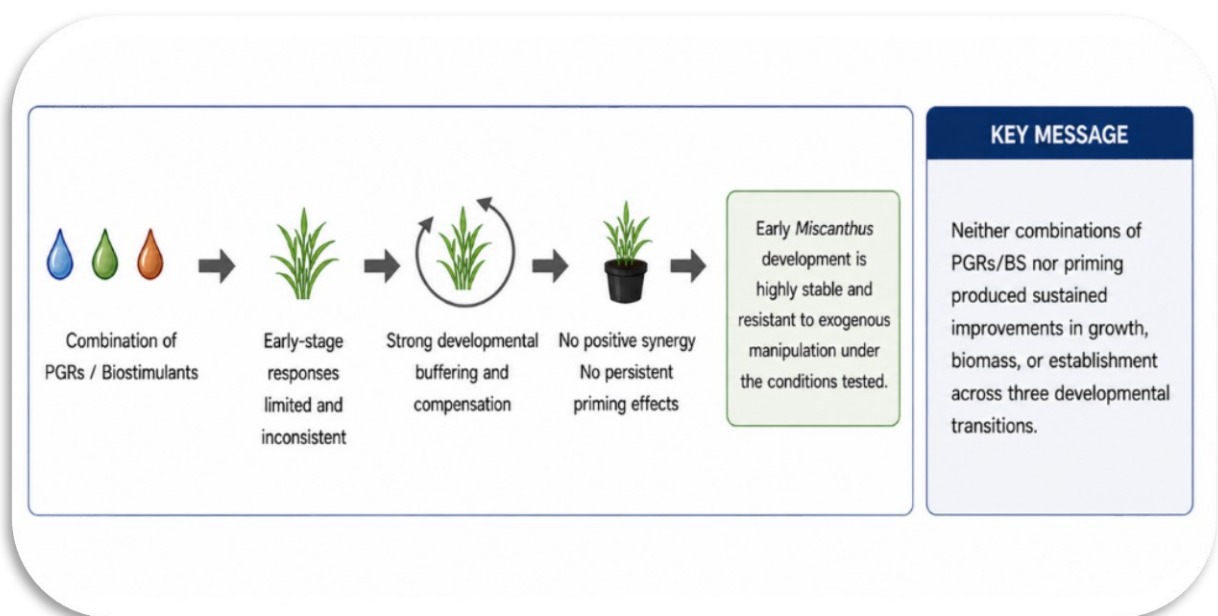


Figure S9. HPLC identification and quantification of carotenoids. Representative high-performance liquid chromatography (HPLC) chromatogram showing the separation and identification of major carotenoids in *Miscanthus* stem extracts, with annotated peaks corresponding to trans-lutein and trans-β-carotene (top panel). Insets show expanded views of the respective chromatographic peaks. Quantification was performed using external calibration with authentic standards. Standard curves for trans-lutein (middle panel) and trans-β-carotene (bottom panel) demonstrate linear relationships between peak area and concentration across the concentration range analysed ($R^2 = 1.00$ for both compounds). These calibration curves were used to calculate carotenoid concentrations in plant samples.

Chapter 6

Limits of Growth Manipulation in Early *Miscanthus* Development: Evaluating Synergy and Priming Effects.



ABSTRACT

This study examines the extent to which early *Miscanthus* development is amenable to exogenous manipulation through synergistic combinations of plant growth regulators (PGR), biostimulants, and short-duration priming treatments. Using a single seed-derived *Miscanthus* hybrid, growth, biomass accumulation, and morphological development were quantified across early establishment, post-transplant growth, and pre-transplant priming contexts. Across all treatments, developmental trajectories were highly conserved, and no evidence of sustained positive synergy or priming-induced enhancement was observed. Where treatment-specific responses occurred, they were limited in magnitude and transient, failing to persist beyond early establishment and indicating compensatory adjustment rather than stable developmental reprogramming. These findings demonstrate that early *Miscanthus* development might be governed by strong endogenous regulatory control, which constrains the reliability and persistence of externally imposed growth interventions under the conditions tested. The results emphasise the context-dependent nature of PGR and priming responsiveness and delineate clear limits to the practical manipulability of early development in perennial grass systems.

Keywords: *Miscanthus*, Plant growth regulators, Biostimulants, Plug priming, Plug establishment, Synergy

INTRODUCTION

The manipulation of plant growth through exogenous inputs rests on an assumption: that developmental outcomes can be predictably modified by intervening in underlying regulatory systems. This assumption underpins much of modern agronomy and plant physiology, where plant growth regulators (PGR), biostimulant (BS), and related inputs are used to influence developmental processes (Davies, 2010). In practice, however, plant growth is not a linear output of individual signals, but an emergent property of highly integrated networks involving hormonal cross-talk, environmental sensing, biochemical and metabolic signalling, and resource allocation (Müller et al., 2015; Pierik et al., 2009). These networks exhibit non-linear dynamics, redundancy, and feedback regulation, which can limit the predictability of externally imposed interventions (Rademacher, 2015). This raises a fundamental question: to what extent are early developmental processes in perennial crops amenable to external manipulation, and under what conditions do they instead exhibit resistance or buffering?

Miscanthus provides a particularly informative system in which to examine this question. As a perennial C₄ grass with strong early vigour and high biomass potential, its establishment phase is both agronomically critical and biologically complex (Heaton et al., 2008; Clifton-Brown et al., 2017). Early growth involves the coordination of meristem activation, tiller initiation, and carbon allocation, all of which are regulated by interacting hormonal pathways and environmental cues (Lewandowski et al., 2003). While these processes present potential targets for intervention, they are also governed by complex endogenous regulatory networks, raising the possibility that attempts to enhance growth may be constrained or attenuated by interactions with intrinsic developmental and physiological processes.

Plant growth regulators and biostimulants are widely used to probe and manipulate these regulatory systems, yet their effects are notoriously context dependent (Davies, 2010). While single compounds can elicit measurable responses, their combined application introduces additional complexity arising from hormonal crosstalk, feedback regulation, and non-linear interactions that often reduce predictability rather than enhancing it (Müller et al., 2015). The concept of synergy, whereby combined treatments produce effects greater than the sum of their individual components, has therefore been

proposed as a route to enhanced growth outcomes (Calvo *et al.*, 2014). However, empirical evidence across plant systems suggests that such interactions are frequently neutral or antagonistic, reflecting the fact that hormonal networks are not necessarily additive systems but tightly regulated circuits capable of absorbing or redirecting perturbations (Rademacher, 2015; Vanstraelen & Benková, 2012; Depuydt & Hardtke, 2011).

A parallel but conceptually distinct approach is provided by priming. Rather than directly stimulating growth, priming seeks to alter the physiological state of the plant, enabling enhanced responsiveness to subsequent stimuli (Conrath, 2009; Hilker *et al.*, 2016). This process may involve epigenetic modification, accumulation of signalling intermediates, or metabolic pre-adjustment, and is inherently conditional, with benefits typically expressed only under stress or challenged conditions (Conrath *et al.*, 2015; Martinez-Medina *et al.*, 2016). As a result, priming occupies a different conceptual space from direct growth regulation, operating through latent potential rather than immediate phenotypic change.

Despite the theoretical appeal of both synergistic PGR/BS interactions and priming strategies, their application in *Miscanthus* remains poorly understood, particularly during early developmental stages. Much of the existing evidence for PGR or BS efficacy is derived from studies focusing on single compounds, short-term responses, or highly controlled environments that may not fully capture the complexity of developmental regulation in perennial systems (Rademacher, 2015). Similarly, while priming has been extensively characterised in model and annual species, its relevance to perennial grasses during early establishment remains unclear (Conrath *et al.*, 2015; Hilker *et al.*, 2016).

This creates a critical gap in understanding. It is not yet clear whether early *Miscanthus* development can be meaningfully enhanced through combined hormonal interventions, nor whether priming treatments can confer advantages that persist beyond initial establishment. More fundamentally, there is a need to distinguish between transient, context-specific responses and robust developmental shifts that are reproducible across growth stages (Poorter *et al.*, 2012; Robson *et al.*, 2012).

The present study addresses these uncertainties by examining the effects of PGR and/or BS combinations (synergy) and priming treatments across plug production and post-transplant. By following plants across these transitions and quantifying growth trajectories, biomass accumulation, and morphological traits, the study explicitly evaluates not only the presence of treatment effects, but also their consistency and persistence.

MATERIALS AND METHODS

Plant material and experimental overview

All experiments were conducted using a single seed-derived *Miscanthus* hybrid, GNT43 (Aphrodite), supplied by Terravesta Ltd (UK). Full information for Aphrodite, a *M. sacchariflorus* × *M. sinensis* hybrid, one of several produced at IBERS, Aberystwyth and trialled in the UK (Shepherd *et al.*, 2023), is available in the CPVO Register. This genotype was selected due to its consistent establishment performance and relevance to the commercial deployment of *Miscanthus*, as it is currently being marketed by Terravesta Ltd.

This chapter integrates two independent but conceptually linked experiments designed to evaluate whether exogenous inputs can enhance early growth beyond baseline performance: (i) a synergy experiment examining combined plant growth regulator (PGR) and biostimulant (BS) applications during early establishment, and (ii) a priming experiment assessing the effect of short-duration pre-transplant treatments on subsequent growth of untreated plugs (plugs without prior PGR or BS treatment).

Both experiments were conducted at Liverpool John Moores University, UK (53°24'45.1" N, 2°58'47.7" W). Early establishment (seed-to-plug phase) was performed under controlled glasshouse conditions, followed by an outdoor pot phase in which plants were exposed to ambient environmental conditions but maintained under regulated water supply.

Experimental structure and design rationale

The study comprised two sequential experimental components:

- **Experiment 1 (Synergy):** Single and combined PGR/BS treatments applied during the seed-to-plug phase (defined as the period from sowing to physiological readiness for transplanting), with a subset of plants transplanted into pots to assess persistence of treatment effects.
- **Experiment 2 (Priming):** Untreated plugs subjected to pre-transplant priming, followed by post-transplant growth assessment.

Experiment 1: Early establishment responses to PGR and BS treatments

Seed-to-plug establishment conditions

Seed sowing, propagation conditions, and early establishment procedures followed those described in Obaje *et al.* (2026), with the exception that a different commercial multipurpose compost (Levington Multipurpose Compost with added John Innes) was used due to material availability, and heated propagation mats (Biogreen, UK) were employed.

Seeds were sown in 12-celled propagation trays (Mixceco) and maintained under controlled glasshouse conditions (~19–20 °C; ~50–65% relative humidity) with a 16 h light / 8 h dark photoperiod supplemented by LED lighting (Kroptek, UK). Humidity lids (supplied with the propagation trays) were used during germination and removed approximately two weeks after seedling emergence. Trays were placed on heated propagation mats, and irrigation was supplied via subirrigation with occasional foliar spray to maintain non-limiting moisture conditions. Three seeds were sown per cell and thinned down to one after successful germination (approximately 2 weeks).

Treatment composition and application protocol

Treatments consisted of single and combined applications of indole-3-acetic acid (IAA; The Plant Growth Company), Liquid Ice (LI; Evoponic Ltd), and Micropull (MP; Ceres Biotics). The treatment structure followed a factorial combination approach, with the three products applied individually and in all possible pairwise and triple combinations, alongside a water control (Ctrl).

Each treatment combination (single, pairwise, and triple) was applied at two concentration levels (full and half of the recommended rate as described in Obaje *et al.*

(2026)), resulting in 12 treatment conditions plus a water control. The full treatment matrix is provided in Table 1. Treatments were arranged as a randomised complete block design (RCBD), with tray means used as the experimental unit ($n = 5$). Treatments were delivered via subirrigation, and application scheduling followed the protocol described in Obaje *et al.* (2026).

Table 1. Treatment structure showing single, double, and triple combinations of Liquid Ice (LI), indole-3-acetic acid (IAA), and Micropull (MP) applied at full or half concentration.

Treatment	Treatment class	*Component Concentration	Concentration Strength
Ctrl	Control	-	-
LI	Single	0.5	Full
IAA	Single	2	Full
MP	Single	1	Full
LI + IAA	Double	LI 0.5 + IAA 2	Full
LI + IAA	Double	LI 0.25 + IAA 1	Half
LI + MP	Double	LI 0.5 + MP 1	Full
LI + MP	Double	LI 0.25 + MP 0.5	Half
IAA + MP	Double	IAA 2 + MP 1	Full
IAA + MP	Double	IAA 1 + MP 0.5	Half
LI + IAA + MP	Triple	LI 0.5 + IAA 2 + MP 1	Full
LI + IAA + MP	Triple	LI 0.25 + IAA 1 + MP 0.5	Half

*All component concentrations are expressed per litre of solution (mL L^{-1}). For combination treatments, values represent the concentration of each component within the same solution.

Growth and physiological measurements

Growth and physiological measurements followed the same protocols described in Obaje *et al.* (2026). Growth was monitored through repeated measurements of stretched height and ligule height. Tiller number was defined as the number of distinct shoots emerging from the base of the plant, each possessing its own leaf whorl. Bud number was recorded as visible axillary buds at the stem base. Leaf number was counted as fully emerged leaves per plant. At harvest, total above- and below-ground biomass were determined following oven drying to constant weight.

Physiological measurements (MultispeQ-derived parameters: LEF, ΦII , ΦNPQ) and pigment indices (chlorophyll, flavonol, anthocyanin, NFI) were obtained using the same instrumentation and protocols detailed in Chapters 4 and 5. Carotenoid extraction and quantification were performed as described in Chapter 5.

Post-transplant growth conditions and carry-over assessment

This phase followed the protocol detailed in Chapter 4 and 5. A subset of plugs from each treatment was transplanted into pots containing topsoil (Wickes, UK). Pots were placed

outdoors on capillary matting within large trays to allow passive subirrigation and were watered using an automated irrigation system three times daily. This system maintained consistent water availability across treatments while exposing plants to ambient environmental conditions.

Plants were arranged in a randomised complete block design. Each block contained two subsamples per treatment, which were averaged prior to data analysis. Post-transplant measurements included stretched height, ligule height, leaf number, and total biomass, following the protocols described in Chapters 4 and 5.

Experiment 2: Pre-transplant priming treatments

Source of plant material for priming

Plugs used in the priming experiment were derived from untreated control plants from the seed-to-plug phase of Experiment 1, ensuring no prior exposure to PGR or BS treatments.

Priming treatment composition and application

Prior to transplanting, plugs were subjected to a 1-hour priming treatment by submerging the root-soil bulk in treatment solutions, following the procedure described in Chapter 5. Concentrations used corresponded to those listed in Table 1 for the single-treatment class.

The priming experiment followed a completely randomised design (CRD) comprising five treatments: a dry control (CTRL (Dry)), in which plugs received no priming or soaking; a wet control (CTRL (Wet)), in which plugs were soaked in water only; and three treatment groups receiving aqueous solutions of IAA, Liquid Ice, or MicroPull. Each treatment consisted of five independent biological replicates ($n = 5$).

Post-priming growth conditions and measurements

Following priming, plugs were transplanted into pots containing topsoil (Wickes, UK) and maintained outdoors under the same conditions as the carry-over assessment phase of Experiment 1.

Growth was monitored through repeated measurements of stretched height. At harvest, morphological endpoints including tiller number, maximum ligule height, growth rate,

leaf number, root:shoot ratio, and biomass were determined following the protocols described in Chapter 5. Regrowth following transplanting was assessed (defined as the time required for visible resumption of shoot growth).

Physiological and pigment-related measurements (Φ II, Φ NPQ, SPAD, AnthM, ChlM, FlvM, NFI) were conducted using the same instrumentation and protocols described in Chapters 4 and 5.

Statistical analysis

All statistical analyses were performed in R (version 4.6.0), following the analytical framework described in previous chapters. For the seed-to-plug phase, tray means were used as the experimental unit. The carry-over assessment phase of Experiment 1 was analysed as a randomised complete block design with four blocks, with subsamples averaged within blocks. Experiment 2 followed a completely randomised design with five independent replicates per treatment.

Repeated-measures data were analysed using linear mixed-effects models, with treatment, time and their interaction specified as fixed effects and experimental unit (tray, block, or replicate as appropriate) included as a random factor. Single time-point variables were analysed using one-way ANOVA, with Tukey's HSD test used for post-hoc comparisons where appropriate. Statistical significance was assessed at $\alpha = 0.05$.

RESULT

All datasets are presented in Figures 1a–b, 2a–b, and 3a–b, with morphology and establishment endpoints summarised in Tables 2–4. Biochemical and physiological measurements are provided in Tables S1–S3 (Supplementary Material).

Early establishment responses to single and combined PGR/biostimulant treatments

Seedlings exhibited a consistent and progressive increase in stretched height from January through April, reflecting normal early establishment dynamics under controlled conditions. As shown in Figure 1a, mean height trajectories for the control, single treatments (IAA, LI, MP), and combination treatments followed closely aligned and near-parallel trends throughout the sampling period. Confidence intervals and

associated error bars overlapped substantially at all time points, and statistical analysis did not detect any treatment effects on stretched height at any stage of development ($p > 0.05$). The absence of separation in growth trajectories indicates that neither individual inputs nor their combinations altered vertical growth dynamics during the seedling-to-plug transition.

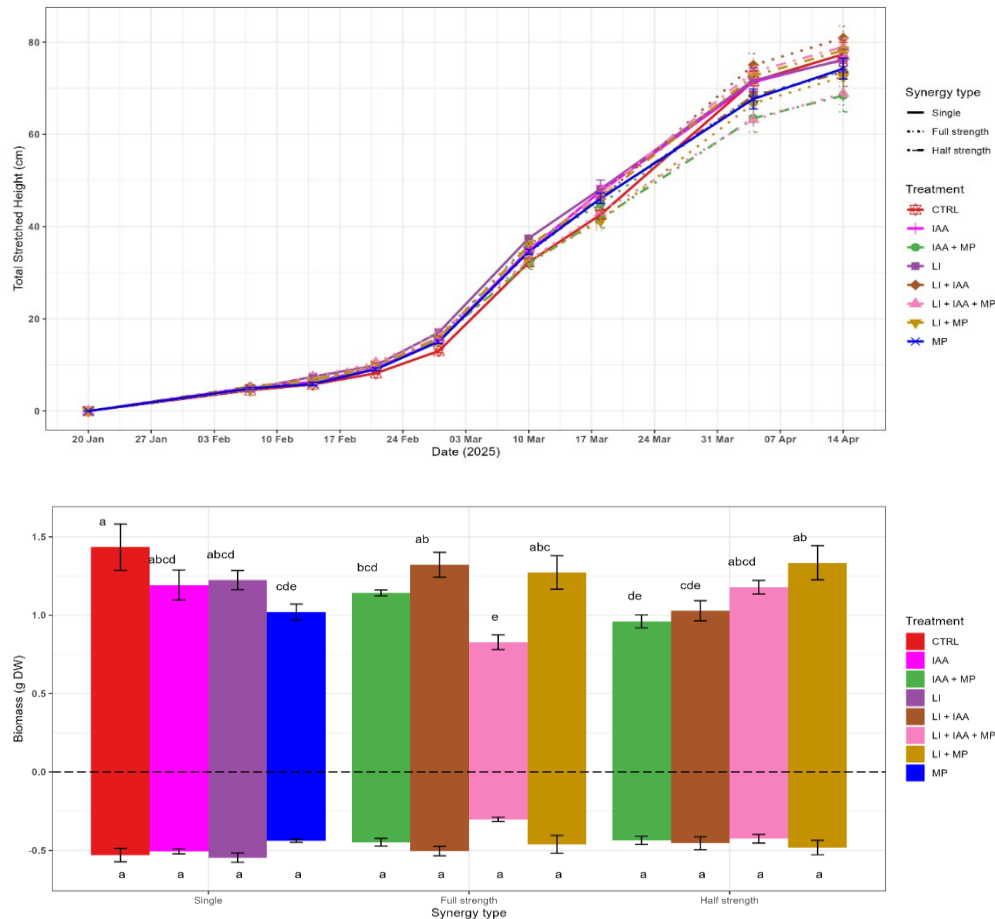


Figure 1. (a) Total stretched height (cm) of *Miscanthus* seedlings from January to April 2025 under individual and combined PGR/BS treatments. Lines represent treatment means $\pm$ SE ($n = 5$). Growth trajectories remained closely aligned across all treatments and synergy types, indicating limited separation during early establishment. **(b).** Total biomass (g DW) at the plug stage, partitioned into above-ground (positive values) and below-ground (negative values) components, with the zero-line marking the division between fractions. Bars represent treatment means $\pm$ SE and are grouped by synergy type (Single, Full, Half). Letters denote statistically distinct groups (Tukey HSD, $\alpha = 0.05$, $n = 5$). A subset of combination treatments showed reduced above-ground biomass, although these differences were not reflected in height trajectories.

At the point of plug harvest, above-ground biomass revealed a more differentiated response among treatments (Figure 1b). While several treatments remained statistically non-significant from the control, a subset of combination treatments produced significantly lower dry biomass ($p < 0.05$). These reductions were treatment-specific rather than systemic across all combinations (see Figure 1b and Table 2 for pairwise groupings). Notably, these biomass reductions were not reflected in the corresponding

height data, indicating a decoupling between vertical extension and biomass accumulation. This pattern suggests that certain treatment combinations may have influenced resource allocation, tissue density, or carbon partitioning without affecting overall plant stature.

Morphological endpoints measured at the plug stage (Table 2) were largely conserved across treatments, reinforcing the limited phenotypic separation observed in growth trajectories. Growth rate estimates, leaf number, tiller number, maximum ligule height, and root:shoot ratio showed similar central tendencies and no statistically significant treatment effects ($p > 0.05$). Bud number represented the only variable with detectable pairwise differences. Post-hoc comparisons revealed a heterogeneous response among the treatment combinations, with several combinations producing fewer buds than the Control, while others produced greater bud numbers than the Control. Differences were also detected between some combination and single-treatment groups, indicating that the response to combined treatments varied according to the specific treatment combination. However, these differences were isolated and not accompanied by consistent variation in either biomass or height, limiting their biological interpretability. Overall, the morphological dataset supports the conclusion that early-stage development was robust to treatment perturbation, with only minor and non-systematic deviations. Full physiological and biochemical outputs for this experiment are reported in Table S1 (Supplementary Material).

Table 2. Morphological and establishment traits during the seed-to-plug stage. Morphological and early establishment traits for *Miscanthus* seedlings following single and combined applications of indole-3-acetic acid (IAA), Liquid Ice (LI), and Micropull (MP) at full or half concentration. Values represent means $\pm$ SE ($n = 5$). Superscript letters denote statistical groupings based on Tukey's HSD ($\alpha = 0.05$). No variables other than bud number exhibited statistically significant treatment effects.

Treatment	Synergy	Growth rate (cm/day)	Number of leaves	Number of buds	Number of tillers	Max ligule height (cm)	Root:shoot ratio
CTRL	Single	0.87 $\pm$ 0.03 ^a	3.05 $\pm$ 0.12 ^a	1.10 $\pm$ 0.16 ^{bcd}	1.07 $\pm$ 0.04 ^a	28.90 $\pm$ 1.70 ^a	0.37 $\pm$ 0.02 ^a
IAA	Single	0.90 $\pm$ 0.01 ^a	2.80 $\pm$ 0.11 ^a	1.80 $\pm$ 0.11 ^a	1.13 $\pm$ 0.10 ^a	27.30 $\pm$ 0.98 ^a	0.43 $\pm$ 0.03 ^a
LI	Single	0.89 $\pm$ 0.03 ^a	3.00 $\pm$ 0.06 ^a	1.67 $\pm$ 0.09 ^{ab}	1.00 $\pm$ 0.00 ^a	27.60 $\pm$ 0.68 ^a	0.45 $\pm$ 0.02 ^a
MP	Single	0.88 $\pm$ 0.02 ^a	2.99 $\pm$ 0.06 ^a	1.10 $\pm$ 0.28 ^{bcd}	1.00 $\pm$ 0.00 ^a	27.90 $\pm$ 1.22 ^a	0.43 $\pm$ 0.02 ^a
LI+IAA	Full	0.93 $\pm$ 0.03 ^a	3.10 $\pm$ 0.07 ^a	1.50 $\pm$ 0.12 ^{abc}	1.03 $\pm$ 0.03 ^a	31.30 $\pm$ 1.18 ^a	0.38 $\pm$ 0.01 ^a
LI+MP	Full	0.84 $\pm$ 0.04 ^a	3.12 $\pm$ 0.05 ^a	0.87 $\pm$ 0.23 ^{de}	1.03 $\pm$ 0.03 ^a	28.40 $\pm$ 1.90 ^a	0.37 $\pm$ 0.04 ^a
IAA+MP	Full	0.86 $\pm$ 0.05 ^a	3.09 $\pm$ 0.11 ^a	0.93 $\pm$ 0.09 ^{cde}	1.00 $\pm$ 0.00 ^a	27.70 $\pm$ 2.66 ^a	0.39 $\pm$ 0.02 ^a
LI+IAA+MP	Full	0.83 $\pm$ 0.03 ^a	3.14 $\pm$ 0.13 ^a	0.73 $\pm$ 0.22 ^e	1.07 $\pm$ 0.04 ^a	25.30 $\pm$ 1.03 ^a	0.37 $\pm$ 0.02 ^a
LI+IAA	Half	0.86 $\pm$ 0.04 ^a	3.03 $\pm$ 0.07 ^a	1.40 $\pm$ 0.19 ^{abcd}	1.00 $\pm$ 0.00 ^a	27.70 $\pm$ 1.22 ^a	0.44 $\pm$ 0.01 ^a
LI+MP	Half	0.91 $\pm$ 0.03 ^a	2.94 $\pm$ 0.13 ^a	1.37 $\pm$ 0.13 ^{abcd}	1.00 $\pm$ 0.00 ^a	30.90 $\pm$ 1.60 ^a	0.37 $\pm$ 0.03 ^a
IAA+MP	Half	0.80 $\pm$ 0.04 ^a	3.22 $\pm$ 0.10 ^a	1.27 $\pm$ 0.13 ^{abcde}	1.00 $\pm$ 0.00 ^a	25.20 $\pm$ 1.37 ^a	0.45 $\pm$ 0.02 ^a
LI+IAA+MP	Half	0.92 $\pm$ 0.02 ^a	3.06 $\pm$ 0.08 ^a	1.30 $\pm$ 0.23 ^{abcde}	1.03 $\pm$ 0.03 ^a	29.60 $\pm$ 1.10 ^a	0.36 $\pm$ 0.06 ^a

Post-transplant growth and persistence of early treatment effects

Following transplant into pots, plants entered a phase of accelerated vegetative growth between May and July, followed by a gradual stabilisation as the growing season progressed. Height trajectories for all treatments are presented in Figure 2a and demonstrate a high degree of concordance across control, single, and combination groups. Mean curves increased in parallel, with substantial overlap in error bars throughout the monitoring period. Statistical analysis confirmed that no treatment effects were detectable for stretched height at any time point or at final measurement ($p > 0.05$). These results indicate that any early-stage treatment effects observed during the plug phase did not propagate into later vertical growth under pot conditions.

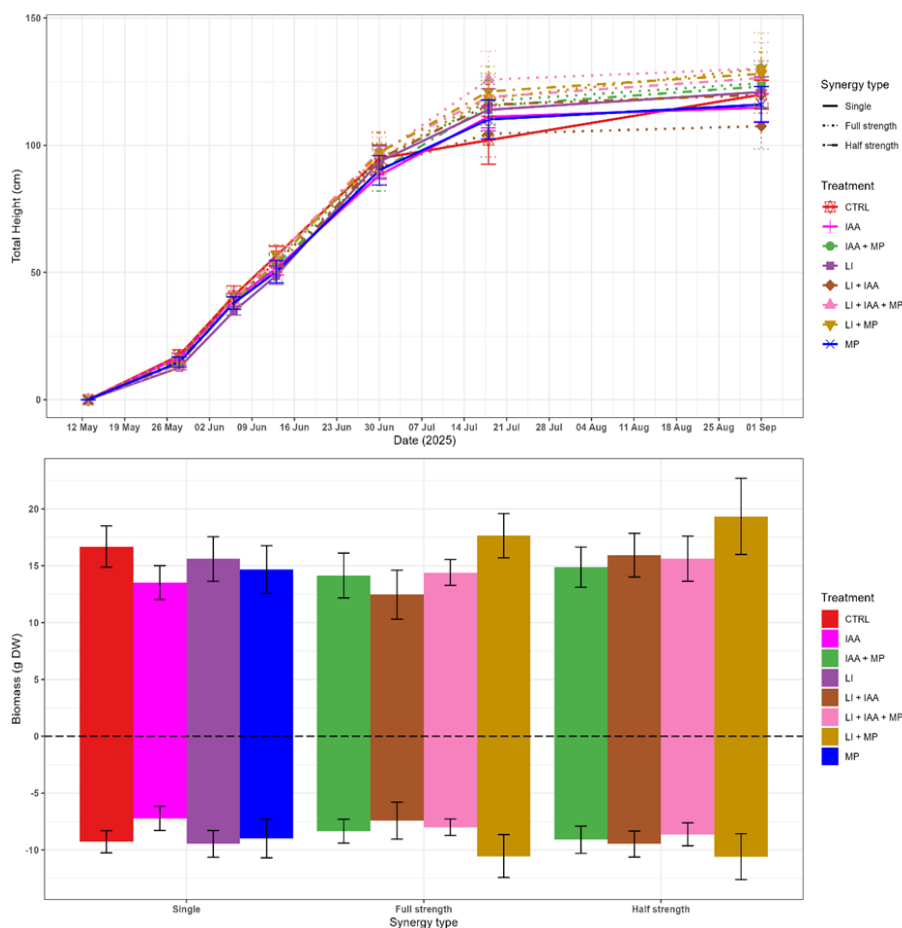


Figure 2. **(a)**. Total plant height (cm) from May to September 2025 following transplant into pots. Treatment means $\pm$ SE ($n = 5$) show parallel and overlapping growth trajectories across all PGR/BS treatments and synergy types, indicating no persistent effects from seed-to-plug treatments. **(b)**. Total biomass (g DW) at pot harvest, partitioned into above-ground (positive) and below-ground (negative) fractions relative to the zero line. Bars represent means $\pm$ SE and are grouped by synergy type; $n = 5$. No statistically significant differences were detected among treatments, and early biomass reductions observed in Experiment 1 did not persist after transplant.

Biomass accumulation at harvest (Figure 2b) further supports this reduced variability of treatment responses. Mean dry weights were comparable across all treatments, with overlapping error bars and no statistically significant differences detected ($p > 0.05$). Importantly, the subset of combination treatments that exhibited reduced biomass at the plug stage did not maintain this effect following transplant. This suggests that the earlier reductions were either transient or compensated during subsequent growth, potentially through recovery mechanisms or environmental buffering under less constrained conditions. The absence of both positive and negative treatment effects at this stage suggests that synergistic interactions, if present, were not sustained across developmental transitions.

Morphological and establishment metrics recorded during the pot phase (Table 3) were similarly uniform across treatments. Days to regrowth, growth rate, leaf number, tiller number, maximum ligule height, and root:shoot ratio all exhibited comparable distributions, and statistical testing did not detect treatment effects for any variable ($p > 0.05$). The consistency across both structural and developmental traits reinforces the conclusion that treatment-induced variation was minimal at this stage. Physiological and biochemical measurements, including MultispeQ outputs and pigment indices, are provided in Table S2 (Supplementary Material) and show no systematic treatment-dependent patterns.

Table 3. Morphological and establishment traits following transplant into pots. Post-transplant growth and morphological traits for *Miscanthus* plants previously exposed to single or combined PGR/biostimulant treatments during the seed-to-plug stage. Values represent means $\pm$ SE ($n = 5$ blocks). Superscript letters indicate Tukey HSD groupings ($\alpha = 0.05$). No significant treatment effects were detected for any variable, indicating reduced variability of growth trajectories after transplant.

Treatment	Synergy	Days to regrowth	Growth rate (cm/day)	Number of leaves	Number of tillers	Max ligule height (cm)	Root:shoot ratio
CTRL	Single	7.38 $\pm$ 1.07 ^a	1.51 $\pm$ 0.06 ^a	5.50 $\pm$ 0.25 ^a	6.75 $\pm$ 0.63 ^a	46.20 $\pm$ 2.02 ^a	0.57 $\pm$ 0.04 ^a
IAA	Single	6.75 $\pm$ 0.48 ^a	1.48 $\pm$ 0.07 ^a	5.38 $\pm$ 0.13 ^a	6.50 $\pm$ 0.98 ^a	40.30 $\pm$ 1.56 ^a	0.53 $\pm$ 0.04 ^a
LI	Single	7.75 $\pm$ 0.48 ^a	1.54 $\pm$ 0.10 ^a	5.62 $\pm$ 0.88 ^a	5.38 $\pm$ 1.05 ^a	45.20 $\pm$ 4.38 ^a	0.63 $\pm$ 0.05 ^a
MP	Single	9.50 $\pm$ 0.84 ^a	1.48 $\pm$ 0.11 ^a	5.50 $\pm$ 0.25 ^a	6.75 $\pm$ 0.78 ^a	46.40 $\pm$ 3.27 ^a	0.59 $\pm$ 0.04 ^a
LI+IAA	Full	6.50 $\pm$ 0.89 ^a	1.45 $\pm$ 0.11 ^a	5.75 $\pm$ 0.00 ^a	5.00 $\pm$ 0.54 ^a	40.10 $\pm$ 4.65 ^a	0.58 $\pm$ 0.04 ^a
LI+MP	Full	7.75 $\pm$ 1.03 ^a	1.65 $\pm$ 0.11 ^a	5.00 $\pm$ 0.25 ^a	6.38 $\pm$ 0.43 ^a	47.50 $\pm$ 6.01 ^a	0.57 $\pm$ 0.04 ^a
IAA+MP	Full	9.12 $\pm$ 1.16 ^a	1.63 $\pm$ 0.09 ^a	5.50 $\pm$ 0.34 ^a	4.50 $\pm$ 0.54 ^a	48.50 $\pm$ 7.38 ^a	0.61 $\pm$ 0.04 ^a
LI+IAA+MP	Full	8.62 $\pm$ 1.31 ^a	1.71 $\pm$ 0.11 ^a	5.50 $\pm$ 0.50 ^a	5.00 $\pm$ 0.71 ^a	49.60 $\pm$ 5.61 ^a	0.57 $\pm$ 0.04 ^a
LI+IAA	Half	7.50 $\pm$ 0.50 ^a	1.52 $\pm$ 0.09 ^a	4.12 $\pm$ 0.13 ^a	5.75 $\pm$ 0.60 ^a	43.10 $\pm$ 2.73 ^a	0.60 $\pm$ 0.04 ^a
LI+MP	Half	7.50 $\pm$ 0.34 ^a	1.65 $\pm$ 0.13 ^a	5.00 $\pm$ 0.00 ^a	4.75 $\pm$ 1.09 ^a	51.40 $\pm$ 5.10 ^a	0.54 $\pm$ 0.04 ^a
IAA+MP	Half	7.00 $\pm$ 0.41 ^a	1.57 $\pm$ 0.12 ^a	5.00 $\pm$ 0.00 ^a	5.12 $\pm$ 0.52 ^a	43.30 $\pm$ 4.01 ^a	0.61 $\pm$ 0.04 ^a
LI+IAA+MP	Half	9.25 $\pm$ 1.97 ^a	1.65 $\pm$ 0.12 ^a	5.25 $\pm$ 0.25 ^a	4.88 $\pm$ 0.90 ^a	47.50 $\pm$ 2.58 ^a	0.57 $\pm$ 0.04 ^a

Growth and establishment responses following pre-transplant priming

Plants subjected to priming treatments following establishment displayed growth dynamics that closely mirrored those observed in the experiment 1. As shown in Figure 3a, stretched height increased in a consistent seasonal pattern, and mean trajectories for all treatments, including CTRL (Dry), CTRL (Wet), and the PGR and BS treatments (IAA, LI, MP), were closely overlaid. Confidence intervals overlapped extensively, and no statistically significant differences in height were detected at any sampling point ($p > 0.05$). These results indicate that priming treatments did not alter above-ground growth trajectories during the monitored period.

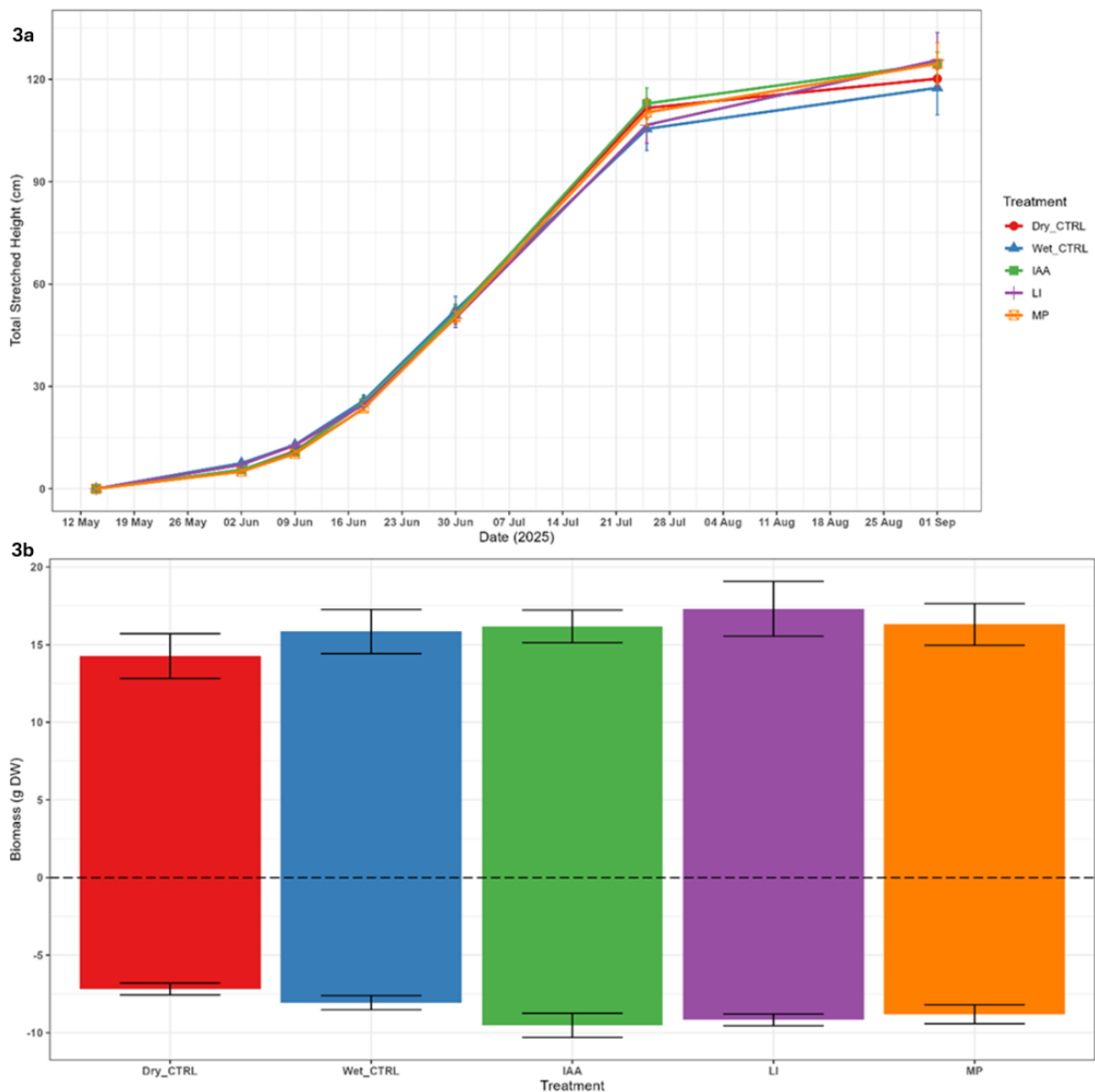


Figure 3. (a) Total stretched height (cm) of primed and control plugs from May to September 2025. All treatments—including Dry CTRL, Wet CTRL, IAA, LI, and MP—showed overlapping growth trajectories with no significant differences

at any time point (means $\pm$ SE, $n = 5$). **(b)** Total biomass (g DW) at harvest, separated into above-ground (positive) and below-ground (negative) components relative to the zero line. Bars show means $\pm$ SE ($n = 5$). Biomass did not differ significantly among treatments, consistent with the absence of treatment effects in height trajectories.

Biomass at priming harvest (Figure 3b) was similarly invariant across treatments. Mean dry weights were comparable, with overlapping error bars and no statistically significant differences among groups ($p > 0.05$). The absence of variation in both growth trajectories and biomass accumulation suggests that priming did not induce measurable changes in overall productivity under the conditions tested.

Morphological and establishment endpoints (Table 4) were largely conserved, although a limited effect was observed for days to regrowth. Specifically, CTRL (Wet) exhibited faster regrowth than at least two priming treatments (IAA and MP), and this pairwise difference reached statistical significance ($p < 0.05$). However, the absence of a significant difference between CTRL (Wet) and CTRL (Dry) indicates that this effect is not consistently attributable to the priming treatments themselves. All other variables, including tiller number, growth rate, leaf number, maximum ligule height, and root:shoot ratio, showed no treatment effects ($p > 0.05$). Physiological and biochemical data are presented in Table S3 (Supplementary Material) and do not reveal consistent treatment-dependent trends.

Table 4. Morphological and regrowth traits following pre-transplant priming. Growth, regrowth timing, and morphological traits for *Miscanthus* plugs subjected to 1-hour priming in water (wet control) or aqueous solutions of IAA, LI, or MP. Values represent means $\pm$ SE ($n = 5$). Superscript letters denote Tukey HSD groupings ($\alpha = 0.05$). Only days to regrowth showed isolated pairwise differences; all other variables were statistically indistinguishable across treatments.

Treatment	Days to regrowth	Number of tillers	Growth rate (cm/day)	Max ligule height (cm)	Number of leaves	Root:shoot ratio
CTRL (Dry)	16.60 $\pm$ 0.60 ^{ab}	6.00 $\pm$ 0.45 ^a	1.23 $\pm$ 0.15 ^a	48.50 $\pm$ 2.06 ^a	4.20 $\pm$ 0.37 ^a	0.52 $\pm$ 0.04 ^a
CTRL (Wet)	14.40 $\pm$ 0.40 ^a	6.60 $\pm$ 0.25 ^a	1.20 $\pm$ 0.13 ^a	47.90 $\pm$ 5.52 ^a	5.20 $\pm$ 0.20 ^a	0.53 $\pm$ 0.06 ^a
IAA	17.40 $\pm$ 1.03 ^b	6.40 $\pm$ 0.60 ^a	1.25 $\pm$ 0.15 ^a	51.00 $\pm$ 2.81 ^a	5.00 $\pm$ 0.32 ^a	0.60 $\pm$ 0.05 ^a
LI	15.20 $\pm$ 0.49 ^{ab}	7.25 $\pm$ 0.56 ^a	1.20 $\pm$ 0.13 ^a	50.80 $\pm$ 4.76 ^a	5.50 $\pm$ 0.58 ^a	0.55 $\pm$ 0.07 ^a
MP	17.80 $\pm$ 0.74 ^b	7.00 $\pm$ 0.45 ^a	1.23 $\pm$ 0.14 ^a	53.40 $\pm$ 3.65 ^a	5.60 $\pm$ 0.25 ^a	0.55 $\pm$ 0.03 ^a

Consistency of growth responses across developmental transitions

Across the two experiments, plant growth, biomass accumulation, and morphological development followed consistent seasonal trajectories that were largely unaffected by treatment. Figures 1a–b, 2a–b, and 3a–b collectively demonstrate that mean values for

control, single, and combination treatments fall within overlapping error ranges for the primary response variables. Statistical analyses consistently failed to detect treatment effects for stretched height, biomass, or key morphological endpoints ($p > 0.05$).

Crucially, no evidence of positive synergy was observed at any stage of development. Combination treatments did not significantly outperform single treatments or controls for any measured outcome. In the seeds-to-plugs phase, some combination treatments showed numerically lower biomass than the Control, but these differences were not statistically significant. These effects were not sustained into later developmental stages, suggesting limited persistence or biological relevance. Similarly, priming treatments did not confer consistent downstream advantages in growth, establishment, or biomass accumulation. Observed effects were sparse, treatment-specific, and not reproducible across related comparisons.

Taken together, these results indicate that under the experimental conditions applied, *Miscanthus* early growth and establishment are characterised by a high degree of developmental stability, with limited responsiveness to both synergistic treatment combinations and priming interventions.

DISCUSSION

Summary of treatment responsiveness during early *Miscanthus* development

This study was designed on the premise that combinations of plant growth regulators (PGR) and/or biostimulants, as well as priming interventions, could enhance early *Miscanthus* development in seed-derived plants. This expectation is supported by plant physiological theory, in which individual PGR influence growth processes and can, under certain conditions, interact to produce amplified or emergent responses (Davies, 2010; Rademacher, 2015; Müller *et al.*, 2015). Similarly, priming is known to modify plant physiological state in ways that enhance subsequent performance, particularly under environmental challenge (Conrath *et al.*, 2015; Hilker *et al.*, 2016; Obaje *et al.*, 2026).

In contrast, the present results demonstrate a consistent absence of treatment responsiveness across all experimental phases. Growth trajectories, biomass accumulation, and morphological traits remained largely invariant across treatments,

with only isolated and transient differences detected. This contrasts with earlier chapters in this thesis, where single PGR and BS applications (e.g. Liquid ice and IAA) produced clear and statistically significant enhancements in early growth. The divergence therefore does not indicate a general lack of treatment efficacy but rather highlights the context-dependent nature of responsiveness, governed by the physiological and environmental conditions under which treatments are applied.

A defining feature of the present experiment is that early establishment occurred under modified conditions, including the use of a different compost substrate and the introduction of a heat mat during the seed-to-plug phase. Although these factors were not explicitly tested as experimental variables, they likely altered the physiological baseline of the system and, consequently, the expression of treatment effects. The results therefore suggest that early *Miscanthus* development can exhibit strong developmental canalisation, whereby endogenous regulatory systems buffer against exogenous perturbation (Müller *et al.*, 2015). Importantly, the contrast with earlier findings indicates that this buffering is conditional rather than absolute, highlighting a key tension between theoretical manipulability and observed robustness in perennial grass development.

Limits to multi-hormonal manipulation during early establishment

The absence of treatment effects on stretched height during the seeds-to-plugs phase indicates that vertical growth remained under strong endogenous control despite the application of multiple PGR and/or BS combinations. Internode elongation in grasses is regulated through tightly coordinated interactions among auxin, gibberellins, and cytokinins, which operate within spatially and temporally constrained gradients (Depuydt & Hardtke, 2014; Müller *et al.*, 2015). Exogenous perturbation of these systems is therefore inherently difficult unless treatments are precisely aligned with developmental windows and applied at sufficient intensity (Rademacher, 2015).

The expectation that combining PGR and/or BS would produce synergistic effects assumes a degree of additivity within these signalling networks. However, plant hormonal systems are characterised by extensive crosstalk and feedback regulation, meaning that combined inputs often converge on shared pathways, resulting in redundancy,

saturation, or antagonism rather than amplification (Davies, 2010; Müller et al., 2015). The absence of positive synergy observed here is therefore consistent with a growing body of evidence indicating that multi-hormonal manipulation does not reliably enhance growth responses, particularly in systems with strong endogenous regulation (Vanstraelen & Benková, 2012; Depuydt & Hardtke, 2011; Rademacher, 2015).

The reductions in seed-to-plug stage biomass observed in specific combination treatments (e.g. full-strength combination of all single treatment) provide a more nuanced indication of treatment interaction. The decoupling between height and biomass suggests that elongation processes remained buffered, while carbon allocation or tissue construction was transiently perturbed. Such responses are consistent with shifts in source–sink dynamics or metabolic allocation, where hormonal imbalance redirects assimilate away from structural biomass (Poorter et al., 2009). In this context, combined treatments may have imposed short-term physiological costs or disrupted regulatory balance, leading to reduced biomass accumulation without affecting elongation.

Importantly, these effects were not sustained beyond the seed-to-plug stage. The rapid reduction in variability of treatments indicates strong compensatory capacity, consistent with the flexible carbon economy and developmental plasticity of perennial grasses (Clifton-Brown & Lewandowski, 2000; Hastings et al., 2009). This reinforces the interpretation that the PGR and/or BS combinations induced transient perturbations rather than stable developmental change.

Failure of early treatment effects to propagate across developmental stages

The absence of treatment effects in the plugs-in-pots phase demonstrates that early-stage perturbations did not propagate across developmental transitions. This finding is particularly important, as it indicates that even where treatments produced measurable effects, these were insufficient to influence subsequent growth trajectories.

As *Miscanthus* plants mature, intrinsic developmental trajectories increasingly dominate, and genotypic differences in traits such as senescence become more stable and pronounced. Consequently, early-stage variation contributes proportionally less to overall biomass outcomes in later growth stages (Robson et al., 2012). In addition,

increased root volume and resource availability reduce sensitivity to prior conditions, promoting reduced variability among treatments (Heaton *et al.*, 2008). The lack of carry-over effects observed here therefore suggests that early-stage hormonal manipulation, at least under the conditions tested, does not translate into meaningful longer-term outcomes.

These findings have important implications for the interpretation of earlier positive results obtained with single treatments. In previous experiments within this research, comparable interventions produced clear and sustained effects that extended beyond early developmental stages. The absence of comparable persistence in the present study does not undermine those earlier observations, but instead highlights the conditional nature of treatment responsiveness.

Context-dependence of priming responses under non-stress conditions

Priming treatments similarly failed to produce consistent effects on growth, biomass, or morphology. This outcome must be interpreted within the theoretical framework of priming, which posits that priming enhances responsiveness to subsequent stress rather than directly promoting growth under optimal conditions (Conrath *et al.*, 2015; Hilker *et al.*, 2016). In the absence of a triggering stress, primed plants are typically phenotypically indistinguishable from controls.

It is important to note that, with the exception of the dry control, all treatments were exposed to water either as a control condition or as the medium for priming. As such, the observed lack of response cannot be attributed to differences in water availability among treatments, but instead reflects a broader absence of priming expression under the conditions tested.

The isolated difference in regrowth time involving the wet control was not supported by consistent patterns across treatments and is therefore unlikely to represent a meaningful priming effect. While water availability can influence regrowth dynamics through effects on turgor and metabolic activation (Parent & Tardieu, 2014), the lack of reproducibility indicates that this observation is context-specific rather than treatment-driven.

CONCLUSION

This chapter demonstrates that early *Miscanthus* development is largely resistant to exogenous manipulation through synergistic PGR and/or BS combinations and priming under the conditions tested. Across all developmental phases, growth and biomass responses were highly conserved, and any treatment effects observed were transient and non-persistent. These findings indicate that endogenous regulatory control dominates early establishment, limiting the scope for reliable growth enhancement at this stage. As such, this chapter defines clear constraints on the practical manipulability of early *Miscanthus* development and establishes a benchmark against which future intervention strategies must be evaluated.

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Supplementary materials

Table S1. Pigment composition and photosynthetic parameters during the seed-to-plug stage.

Mean $\pm$ SE (n = 5) values for lutein, α -carotene, β -carotene, effective quantum yield of PSII (Phi2), non-photochemical quenching yield (PhiNPQ), and SPAD chlorophyll index in *Miscanthus* seedlings following single and combined PGR/biostimulant treatments. Superscript letters indicate Tukey HSD groupings (α = 0.05). No consistent treatment-dependent trends were detected.

Treatment	Synergy	Lutein	α -carotene	β -carotene	Phi2	PhiNPQ	SPAD
CTRL	Single	21.50 $\pm$ 1.41 ^{ab}	3.92 $\pm$ 0.15 ^a	13.60 $\pm$ 1.03 ^{ab}	0.25 $\pm$ 0.07 ^{cde}	0.63 $\pm$ 0.09 ^{abc}	16.10 $\pm$ 1.87 ^{ab}
IAA	Single	19.30 $\pm$ 1.66 ^{abc}	3.67 $\pm$ 0.06 ^a	11.20 $\pm$ 0.73 ^{ab}	0.45 $\pm$ 0.02 ^{ab}	0.41 $\pm$ 0.03 ^{bcd}	15.40 $\pm$ 0.35 ^{ab}
LI	Single	19.40 $\pm$ 0.88 ^{abc}	3.72 $\pm$ 0.09 ^a	12.30 $\pm$ 0.42 ^{ab}	0.18 $\pm$ 0.01 ^{de}	0.76 $\pm$ 0.02 ^a	15.40 $\pm$ 0.28 ^{ab}
MP	Single	17.00 $\pm$ 0.44 ^{bc}	3.67 $\pm$ 0.10 ^a	11.10 $\pm$ 0.36 ^{ab}	0.22 $\pm$ 0.03 ^{cde}	0.69 $\pm$ 0.03 ^{ab}	15.50 $\pm$ 0.40 ^{ab}
LI+IAA	Full	18.50 $\pm$ 1.64 ^{bc}	3.62 $\pm$ 0.14 ^a	11.80 $\pm$ 0.92 ^{ab}	0.40 $\pm$ 0.02 ^{abc}	0.42 $\pm$ 0.05 ^{cd}	18.50 $\pm$ 0.46 ^a
LI+MP	Full	19.10 $\pm$ 1.32 ^{abc}	3.86 $\pm$ 0.10 ^a	12.70 $\pm$ 0.64 ^{ab}	0.35 $\pm$ 0.06 ^{bcd}	0.53 $\pm$ 0.08 ^{abcd}	15.40 $\pm$ 0.71 ^{ab}
IAA+MP	Full	15.80 $\pm$ 2.09 ^c	3.41 $\pm$ 0.12 ^a	09.82 $\pm$ 0.86 ^b	0.57 $\pm$ 0.03 ^a	0.33 $\pm$ 0.09 ^d	13.10 $\pm$ 0.70 ^b
LI+IAA+MP	Full	23.70 $\pm$ 2.17 ^a	4.12 $\pm$ 0.22 ^a	15.00 $\pm$ 1.36 ^a	0.29 $\pm$ 0.01 ^{bcde}	0.59 $\pm$ 0.01 ^{abc}	19.60 $\pm$ 0.46 ^a
LI+IAA	Half	20.20 $\pm$ 1.44 ^{abc}	3.91 $\pm$ 0.24 ^a	13.10 $\pm$ 0.86 ^{ab}	0.17 $\pm$ 0.02 ^e	0.73 $\pm$ 0.02 ^a	16.90 $\pm$ 0.71 ^{ab}
LI+MP	Half	18.90 $\pm$ 1.83 ^{abc}	3.77 $\pm$ 0.14 ^a	12.70 $\pm$ 1.34 ^{ab}	0.53 $\pm$ 0.01 ^a	0.28 $\pm$ 0.01 ^d	16.20 $\pm$ 1.15 ^{ab}
IAA+MP	Half	19.50 $\pm$ 2.06 ^{abc}	3.89 $\pm$ 0.26 ^a	13.10 $\pm$ 1.63 ^{ab}	0.19 $\pm$ 0.01 ^{de}	0.72 $\pm$ 0.02 ^a	16.10 $\pm$ 0.89 ^{ab}
LI+IAA+MP	Half	19.20 $\pm$ 1.33 ^{abc}	3.81 $\pm$ 0.15 ^a	12.50 $\pm$ 0.69 ^{ab}	0.27 $\pm$ 0.01 ^{bcde}	0.63 $\pm$ 0.01 ^{abc}	20.20 $\pm$ 0.45 ^a

Table S2. Physiological and pigment-related parameters during plugs-in-pots stage.

Mean $\pm$ SE (n = 5) values for Phi2, PhiNPQ, SPAD, anthocyanin (AnthM), chlorophyll (ChlM), flavonol (FlvM), and nitrogen balance index (NFI) measured in *Miscanthus* plants previously exposed to single or combined PGR/biostimulant treatments. Superscript letters denote Tukey HSD groupings (α = 0.05). All variables were statistically indistinguishable across treatments, indicating reduced variability of physiological status after transplant.

Treatment	Synergy	Phi2	PhiNPQ	SPAD	AnthM	ChlM	FlvM	NFI
CTRL	Single	0.47 $\pm$ 0.06 ^a	0.31 $\pm$ 0.07 ^a	28.70 $\pm$ 1.08 ^a	0.06 $\pm$ 0.01 ^a	0.47 $\pm$ 0.04 ^a	1.12 $\pm$ 0.07 ^a	0.44 $\pm$ 0.05 ^a
IAA	Single	0.35 $\pm$ 0.02 ^a	0.42 $\pm$ 0.06 ^a	28.40 $\pm$ 0.30 ^a	0.07 $\pm$ 0.01 ^a	0.49 $\pm$ 0.03 ^a	1.08 $\pm$ 0.02 ^a	0.49 $\pm$ 0.04 ^a
LI	Single	0.44 $\pm$ 0.02 ^a	0.30 $\pm$ 0.04 ^a	27.60 $\pm$ 2.71 ^a	0.09 $\pm$ 0.01 ^a	0.49 $\pm$ 0.07 ^a	1.16 $\pm$ 0.10 ^a	0.43 $\pm$ 0.04 ^a
MP	Single	0.50 $\pm$ 0.03 ^a	0.29 $\pm$ 0.04 ^a	25.40 $\pm$ 3.21 ^a	0.07 $\pm$ 0.02 ^a	0.43 $\pm$ 0.06 ^a	1.13 $\pm$ 0.05 ^a	0.41 $\pm$ 0.09 ^a
LI+IAA	Full	0.51 $\pm$ 0.03 ^a	0.26 $\pm$ 0.04 ^a	27.50 $\pm$ 1.50 ^a	0.07 $\pm$ 0.01 ^a	0.49 $\pm$ 0.03 ^a	1.08 $\pm$ 0.01 ^a	0.49 $\pm$ 0.02 ^a
LI+MP	Full	0.49 $\pm$ 0.02 ^a	0.23 $\pm$ 0.00 ^a	24.10 $\pm$ 2.50 ^a	0.09 $\pm$ 0.02 ^a	0.44 $\pm$ 0.04 ^a	1.14 $\pm$ 0.09 ^a	0.41 $\pm$ 0.05 ^a
IAA+MP	Full	0.46 $\pm$ 0.03 ^a	0.35 $\pm$ 0.04 ^a	25.40 $\pm$ 1.58 ^a	0.07 $\pm$ 0.00 ^a	0.43 $\pm$ 0.04 ^a	1.13 $\pm$ 0.03 ^a	0.40 $\pm$ 0.04 ^a
LI+IAA+MP	Full	0.45 $\pm$ 0.05 ^a	0.34 $\pm$ 0.08 ^a	25.80 $\pm$ 1.78 ^a	0.08 $\pm$ 0.02 ^a	0.47 $\pm$ 0.02 ^a	1.18 $\pm$ 0.05 ^a	0.41 $\pm$ 0.03 ^a
LI+IAA	Half	0.38 $\pm$ 0.04 ^a	0.44 $\pm$ 0.07 ^a	23.30 $\pm$ 1.39 ^a	0.13 $\pm$ 0.02 ^a	0.42 $\pm$ 0.02 ^a	1.25 $\pm$ 0.05 ^a	0.34 $\pm$ 0.03 ^a
LI+MP	Half	0.30 $\pm$ 0.06 ^a	0.58 $\pm$ 0.07 ^a	21.30 $\pm$ 3.67 ^a	0.12 $\pm$ 0.02 ^a	0.43 $\pm$ 0.05 ^a	1.30 $\pm$ 0.08 ^a	0.33 $\pm$ 0.05 ^a
IAA+MP	Half	0.49 $\pm$ 0.03 ^a	0.29 $\pm$ 0.04 ^a	28.00 $\pm$ 2.18 ^a	0.08 $\pm$ 0.03 ^a	0.45 $\pm$ 0.04 ^a	1.20 $\pm$ 0.06 ^a	0.40 $\pm$ 0.05 ^a
LI+IAA+MP	Half	0.41 $\pm$ 0.06 ^a	0.42 $\pm$ 0.07 ^a	27.10 $\pm$ 1.57 ^a	0.09 $\pm$ 0.03 ^a	0.44 $\pm$ 0.03 ^a	1.21 $\pm$ 0.07 ^a	0.37 $\pm$ 0.04 ^a

Table S3. Physiological and pigment-related parameters following pre-transplant priming.

Mean $\pm$ SE (n = 5) values for Phi2, PhiNPQ, SPAD, AnthM, ChlM, FlvM, and NFI in *Miscanthus* plugs subjected to 1-hour priming in water (wet control) or aqueous solutions of IAA, LI, or MP. Superscript letters denote Tukey HSD groupings (α = 0.05). No significant treatment effects were detected for any variable, consistent with the absence of priming-induced physiological change under non-stress conditions.

Treatment	Phi2	PhiNPQ	SPAD	AnthM	ChlM	FlvM	NFI
CTRL (Dry)	0.45 $\pm$ 0.05 ^a	0.31 $\pm$ 0.06 ^a	18.70 $\pm$ 1.18 ^a	0.14 $\pm$ 0.02 ^a	0.38 $\pm$ 0.03 ^a	1.21 $\pm$ 0.12 ^a	0.33 $\pm$ 0.06 ^a
CTRL (Wet)	0.54 $\pm$ 0.04 ^a	0.26 $\pm$ 0.04 ^a	19.00 $\pm$ 1.47 ^a	0.16 $\pm$ 0.04 ^a	0.33 $\pm$ 0.03 ^a	1.19 $\pm$ 0.06 ^a	0.29 $\pm$ 0.04 ^a
IAA	0.50 $\pm$ 0.02 ^a	0.24 $\pm$ 0.03 ^a	24.30 $\pm$ 1.11 ^a	0.11 $\pm$ 0.01 ^a	0.46 $\pm$ 0.02 ^a	1.21 $\pm$ 0.03 ^a	0.38 $\pm$ 0.02 ^a
LI	0.41 $\pm$ 0.04 ^a	0.28 $\pm$ 0.02 ^a	21.70 $\pm$ 2.01 ^a	0.11 $\pm$ 0.01 ^a	0.41 $\pm$ 0.04 ^a	1.33 $\pm$ 0.02 ^a	0.31 $\pm$ 0.04 ^a
MP	0.43 $\pm$ 0.06 ^a	0.27 $\pm$ 0.07 ^a	21.30 $\pm$ 1.33 ^a	0.14 $\pm$ 0.02 ^a	0.38 $\pm$ 0.03 ^a	1.20 $\pm$ 0.14 ^a	0.36 $\pm$ 0.09 ^a

Table S4. Statistical summary of treatment, time (date), and treatment × time interaction effects on measured traits across three experimental stages (seeds-to-plugs, plugs-in-pots, and plug priming). Values represent p-values obtained from ANOVA (single time-point variables) or linear mixed-effects models (repeated measures). Significant effects ($p < 0.05$) are indicated by an asterisk (*).

Experiment/stage	Trait	Treatment	Date	Interaction
Seeds-to-plugs	Stretched height	0.465	<0.001*	0.348
	Above ground biomass	<0.001*		
	Below ground biomass	0.332		
	Growth rate	0.212		
	Number of leaves	0.928		
	Number of buds	<0.001*		
	Number of tillers	0.998		
	Max ligule height	0.086		
	Root:shoot ratio	0.059		
	Lutein	<0.001*		
	α-carotene	1.000		
	β-carotene	0.021*		
	Phi2	0.031*		
	PhiNPQ	0.027*		
	SPAD	<0.001*		
Plugs-in-pots	Stretched height	0.112	<0.001*	0.998
	Above ground biomass	0.055		
	Below ground biomass	0.055		
	Days to regrowth	0.426		
	Growth rate	0.457		
	Number of leaves	0.081		
	Number of tillers	0.333		
	Max ligule height	0.726		
	Root:shoot ratio	0.890		
	Phi2	0.344		
	PhiNPQ	0.434		
	SPAD	0.443		
	AnthM	0.997		
	ChlM	0.960		
	FlvM	0.059		
	NFI	0.327		
Plug priming	Stretched height	0.954	<0.001*	0.985
	Above ground biomass	0.689		
	Below ground biomass	0.053		
	Days to regrowth	0.010*		
	Growth rate	0.998		
	Max ligule height	0.864		
	Number of tillers	0.474		
	Number of leaves	0.083		
	Root:shoot ratio	0.057		
	Phi2	0.945		
	PhiNPQ	0.978		
	SPAD	0.997		
	AnthM	0.955		
	ChlM	0.621		
	FlvM	0.380		
	NFI	0.780		

Chapter 7

General Discussion, Conclusion and Recommendations



GENERAL DISCUSSION

This thesis demonstrated that early development in *Miscanthus* can be manipulated through the application of plant growth regulators (PGR) and biostimulants (BS), although the magnitude, direction and persistence of these responses were strongly dependent on treatment, genotype, developmental stage and environmental context. Across the experimental chapters, PGR and BS treatments produced measurable changes in early growth, physiological traits and biomass allocation, demonstrating that the establishment phase represents a genuine window of developmental responsiveness. However, these effects were not uniformly maintained following transplanting or under field conditions, indicating that early-stage interventions provide leverage over development rather than deterministic control.

The scoping review (Chapter 2) provided the context for these findings by showing that the existing evidence base for biological interventions in *Miscanthus* is concentrated in controlled environments, short-duration studies and rhizome-based planting material. Seed-derived and plug-based systems, which are increasingly relevant to scalable establishment, remain comparatively underrepresented (Ashman *et al.*, 2023; Awty-Carroll *et al.*, 2023). This evidence gap provided the rationale for the experimental chapters, which examined whether the plug-production and establishment phase could be used as a physiological window for manipulating early development and whether treatment responses were maintained across developmental stages and environments.

Across Chapters 3 to 5, the experiments demonstrated that early development in *Miscanthus* is indeed manipulatable. PGR and BS applied during plug production produced measurable shifts in shoot growth, pigment composition, and chlorophyll fluorescence. These responses demonstrate that the early ontogenetic stage represents a phase of developmental sensitivity in which external interventions can alter growth trajectories. This aligns with established plant physiological theory, which recognises early development as a period of considerable developmental plasticity (Davies, 2010; Depuydt & Hardtke, 2011). The physiological measurements collected here, particularly chlorophyll fluorescence and pigment ratios, also provided evidence that treatment responses were not restricted to morphological traits, although the mechanisms underlying these responses were not directly established.

However, the experiments also revealed that this manipulability is bounded and strongly conditioned by environmental context. Treatment effects that were clear in controlled environments often attenuated after transplanting and became increasingly difficult to detect under field condition (Chapter 4). This attenuation reflects the transition from internally regulated developmental processes to externally constrained growth environments, where soil structure, moisture dynamics, temperature fluctuations, and microclimatic heterogeneity exert stronger influence on plant performance than the physiological adjustments induced during plug production (Lewandowski *et al.*, 2016; Pidlisnyuk *et al.*, 2020). The field experiment in Chapter 4 illustrated this clearly: treatments that produced measurable physiological or morphological responses in pots did not consistently translate into field-level differences, demonstrating the dominance of environmental signals.

The persistence experiments further reinforced this interpretation. Although some early-stage responses persisted briefly after transplanting, they rarely endured across a full growing season. This pattern reflects the perennial nature of *Miscanthus*, where long-term performance depends on processes such as root system establishment, carbohydrate reserve formation, and overwintering success, processes that are less responsive to short-duration interventions (Clifton-Brown *et al.*, 2019). Early stimulation, therefore, does not guarantee durable improvements, and the assumption that early gains will translate into long-term yield advantages is not supported by the evidence presented here.

Chapter 6 provided critical insight into the limits of early-stage manipulability. Unlike the responses observed in the preceding experimental chapters (Chapter 3-5), treatment effects in Chapter 6 were not consistently detectable under the conditions tested. Rather than representing a contradiction of the earlier findings, this result reinforces the conditional nature of intervention efficacy. This interpretation is also consistent with the evidence identified in the scoping review (Chapter 2), in which positive responses to PGR and BS were frequently reported in environmentally challenged systems, including marginal or contaminated soils. Such conditions may provide a greater physiological constraint against which an intervention can exert a measurable effect, although the available evidence does not establish that environmental stress is necessary for PGR or

BS efficacy. The absence of a detectable response in Chapter 6 therefore demonstrates that treatment effects cannot be assumed to occur independently of environmental and physiological context. Divergent responses across the experimental chapters, where treatments produced positive effects in one context and negligible or negative effects in another, are therefore better interpreted as expressions of context-dependent responsiveness rather than as evidence of uniformly effective or ineffective treatments. The threshold experiments further demonstrated that some treatments exhibit narrow effective ranges, with diminishing returns or counterproductive effects depending on concentration. This underscores the need for dose-specific optimisation rather than generalised application.

Taken together, the findings across this thesis support a conceptual reframing of early-stage intervention. Early development in *Miscanthus* is a manipulatable phase, but manipulability is constrained by environmental signals, developmental inertia, and the inherent variability of biological systems. PGR and BS can influence early growth and physiology, but their effects are conditional, context-dependent, and not consistently maintained when assessed through subsequent morphological or biomass responses. However, attenuation of morphological treatment differences does not necessarily indicate complete loss of physiological effects. The changes in pigment composition and chlorophyll fluorescence observed in this thesis suggest that interventions may influence physiological processes that are not fully expressed as differences in growth under the conditions tested. Such effects could potentially become more apparent when plants subsequently encounter environmental stress, but this possibility was not directly tested in the present study. The establishment phase thus emerges as a constrained control point: a phase where interventions can exert influence, but only within the limits imposed by environmental conditions and the plant's developmental architecture. This reframing moves the field beyond binary interpretations of success and failure and towards a more nuanced understanding of when, where, and to what extent early-stage interventions can meaningfully alter establishment trajectories.

CONCLUSIONS

This thesis leads to several overarching conclusions:

1. **The existing evidence base is structurally biased**, with controlled environments and short-term studies overestimating treatment efficacy relative to real-world conditions.
2. **Early development represents a critical control point in *Miscanthus* establishment**, with the potential to exert disproportionate influence on subsequent growth, productivity, and stand performance.
3. **Plant growth regulators and biostimulants can manipulate early development**, but their effects operate within narrow and context-dependent limits shaped by environmental and developmental constraints.
4. **Early morphological gains were not consistently maintained after transplanting or under field conditions**, demonstrating that short-duration interventions cannot reliably overcome external constraints under the conditions tested. However, persistence of treatment-induced physiological changes and their potential contribution to stress resilience remain unresolved.

Together, these findings support a bounded, conditional model of early-stage manipulability, in which interventions offer leverage but not deterministic control.

RECOMMENDATIONS AND PRACTICAL APPLICATION

1. **Future research should prioritise multi-year, field-based studies** that track the persistence of early-stage interventions across complete establishment cycles and subsequent growing seasons. Short-term responses observed during plug production or the first growing season should not be assumed to translate into sustained improvements in biomass production. Longer-term studies should therefore assess establishment, overwinter survival, biomass accumulation, biomass allocation and treatment persistence across multiple seasons.
2. **Integrated experimental designs are needed** that combine growth, physiological and environmental measurements to provide a more coherent understanding of how PGR and BS influence early development. Measurements of plant growth should be considered alongside physiological indicators such as chlorophyll fluorescence and pigment composition, together with environmental

variables including soil moisture, temperature and nutrient availability. Where possible, direct measurements of endogenous hormones and other relevant biochemical responses would help distinguish physiological mechanisms from phenotypic responses.

- 3. Dose–response relationships should be investigated more systematically** to identify effective application ranges and potential thresholds for individual PGR and BS treatments. The results of this thesis indicate that longer or greater exposure does not necessarily produce greater responses and may result in diminished or contrasting effects. Future studies should therefore evaluate treatment concentration, frequency and duration together to identify practical application windows and minimise counterproductive applications.
- 4. Variability and non-response should be treated as informative outcomes**, rather than simply as failures of an intervention. Differences among hybrids, treatments and environments can help identify the conditions under which biological interventions are most likely to produce measurable responses. Future studies should therefore explicitly investigate genotype × treatment and treatment × environment interactions, including cases where treatment effects are attenuated or absent.
- 5. Research should expand its focus on seed- and plug-based systems**, which remain comparatively underrepresented despite their relevance to scalable deployment of seed-propagated *Miscanthus*. Further work should examine plug size, seedling age, substrate, hardening, planting date, establishment conditions and treatment combinations alongside PGR and BS applications. Optimisation of these factors is particularly important because plug production provides an opportunity to manipulate plant development before field transplantation. Evidence from seed-derived plug studies also indicates that planting date and plug characteristics can influence subsequent establishment and yield.
- 6. For the biomass industry, PGR and biostimulants should be regarded as supplementary, context-dependent tools** within plug production and establishment, rather than as substitutes for appropriate agronomic

management. Their application may provide an opportunity to modify early plant development and potentially improve establishment characteristics under suitable conditions, but treatment responses are unlikely to compensate consistently for inappropriate planting conditions, poor site preparation, water limitation, unsuitable genotype or other constraints encountered after field transplantation. Consequently, commercial application should consider the target genotype, treatment, developmental stage, application duration and anticipated field conditions rather than assuming a universal treatment effect. This is consistent with evidence that planting date, plug characteristics and field establishment conditions can substantially influence subsequent *Miscanthus* performance.

7. **Interventions should be integrated with broader agronomic practices,** including genotype selection, optimisation of planting material and planting date, plug production and hardening, weed management, soil and water management, and site-specific establishment practices. PGR and BS treatments should therefore be evaluated as components of an establishment strategy rather than as isolated interventions. This integrated approach is particularly relevant for deployment on marginal or heterogeneous land, where environmental constraints can strongly influence establishment success. Research on *Miscanthus* establishment has similarly emphasised the interaction between planting material, planting conditions and site characteristics.

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