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

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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—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.

1 | 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. 2009; 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, Wilson, et al. 2023).

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, Awty-Carroll, et al. 2023). 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, Awty-Carroll, et al. 2023). 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 (Pietrini et al. 2011; Szpunar-Krok et al. 2024). 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 10 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.

2 | Materials and Methods

2.1 | Plant Materials and Growth Conditions

The plant materials used in this study were three seed-based *Miscanthus × giganteus* J.M. Greef & Deuter ex Hodk. & Renvoize hybrids (*M. sacchariflorus x 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-h 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

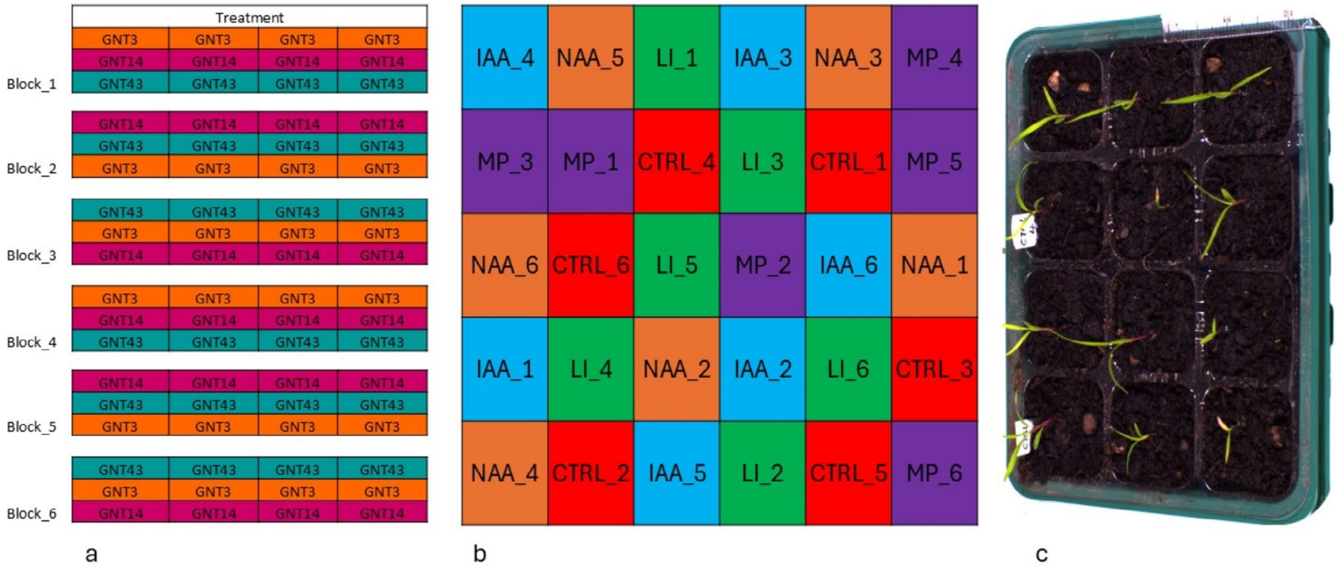

FIGURE 1 | Panel (a) shows the layout of hybrids within each block of treatment; panel (b) maps the specific PGR treatment and replicate number to individual tray starting positions; panel (c) is a representative photograph of one complete block of the 12-celled trays.

were sown on January 26, 2024, and the lids (supplied with the trays) were left on for 2 weeks after sowing to maintain a humid environment, which is essential for *Miscanthus* seed germination.

2.2 | 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, trays were repositioned weekly, ensuring more uniform exposure to light and temperature across treatments rather than maintaining a fixed spatial block structure.

2.3 | 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 1-week preliminary seed germination test and 6 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 Supporting Information (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 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 10 weeks, after which all trays received only water for the remainder of the study (6 more weeks).

2.4 | Data Collection and Measurements

2.4.1 | 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.

2.4.2 | Photosynthesis

Photosynthetic parameters were assessed using a FluorCam 800MF imaging fluorometer (Photon Systems Instruments, Drásov, Czech Republic) 2 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 min before the quenching kinetic analysis to ensure maximum photochemical efficiency of photosystem II (PSII) (Müller and Munné-Bosch 2021; Murchie and Lawson 2013).

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

2.4.3 | Final Harvest Metrics

At experiment's end, fresh above- and below-ground biomass was 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.

2.4.4 | Germination Assessments

Monitored every other day after sowing, days to germination were measured and final percentage germination was taken after 14 days. Germination was defined as the visible emergence of a green shoot from the seed. Percentage germination (PG) was calculated as: PG (%) = (number of germinated seeds/total number of seeds sown per treatment) × 100.

2.5 | 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 and Weisberg 2019). All predictors (PGR, hybrid, and date) had GVIF^(1/(2 × 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 and Cadima 2016; Poorter et al. 2012). Treatment groupings were visualised using 95% confidence ellipses.

3 | Results

3.1 | Germination Response to Plant Growth Regulators

The effects of plant growth regulators (PGRs) 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 PGRs ($p < 0.05$), suggesting a possible negative effect on seed viability or early seedling development in this hybrid.

Overall, while most PGRs 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.

3.2 | Total Height and Growth Dynamics

The application of PGRs noticeably enhanced stem elongation in all three hybrids (Figure 2). Treated seedlings diverged from controls soon after the first application and maintained this advantage beyond the cessation of treatment (vertical dashed line). By the final measurement (14 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× the height of controls. IAA and NAA treatments also increased

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. 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, $n=6$.

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

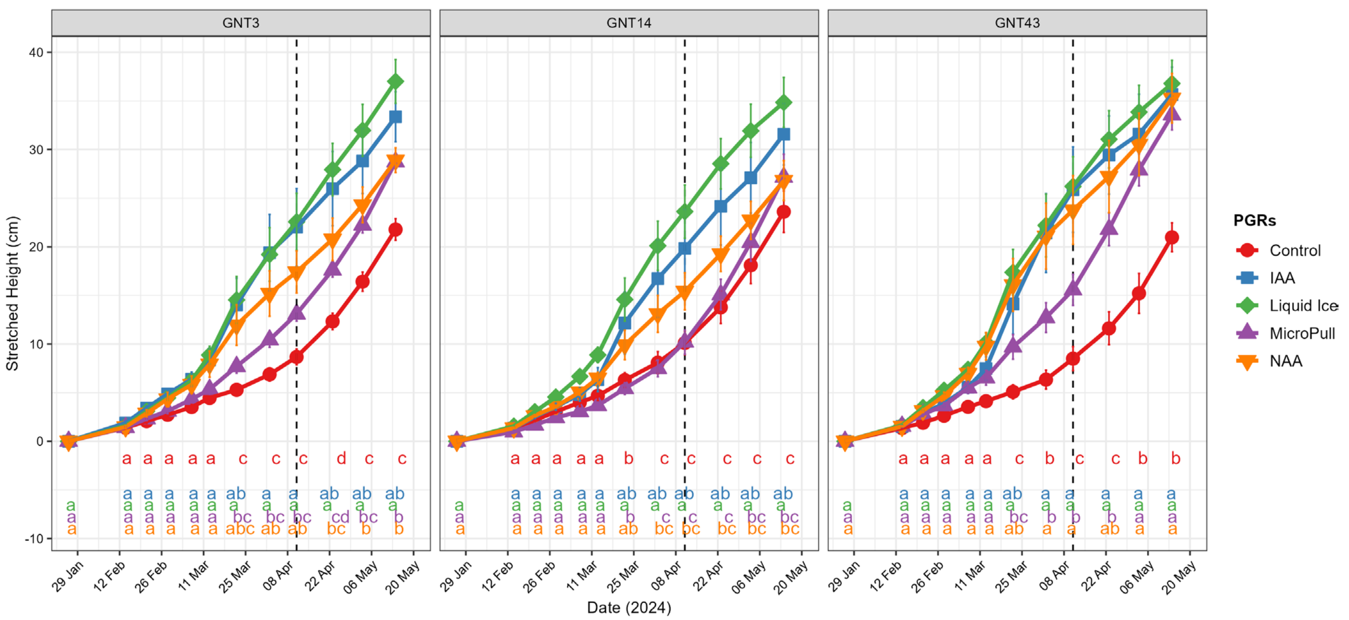

FIGURE 2 | Stretched height of Miscanthus plug under PGR treatments. Weekly measurements of stretched height (cm) from 14 February to 14 May 2024 for hybrids GNT14, GNT3, and GNT43. Solid lines represent the period of PGR application, the vertical dashed line marks treatment cessation; data are mean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

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; Tukey's HSD, $p<0.05$). In GNT43, Liquid Ice, MicroPull, IAA, and NAA all yielded elevated rates (~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.

3.3 | 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.

TABLE 2 | Effects of plant growth regulator treatments on ligule height (LH, cm), growth rate (GR, cm/day), stem diameter (SD, cm^2), number of roots (NR), number of tillers (NT), number of buds (NB), and Root: shoot ratio (RS) in *Miscanthus* hybrids. 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, $n = 6$.

Hybrid	PGR	LH	GR	SD	NR	NT	NB	RS
	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}
GNT3	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}
	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}
GNT14	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}
	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}
GNT43	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}

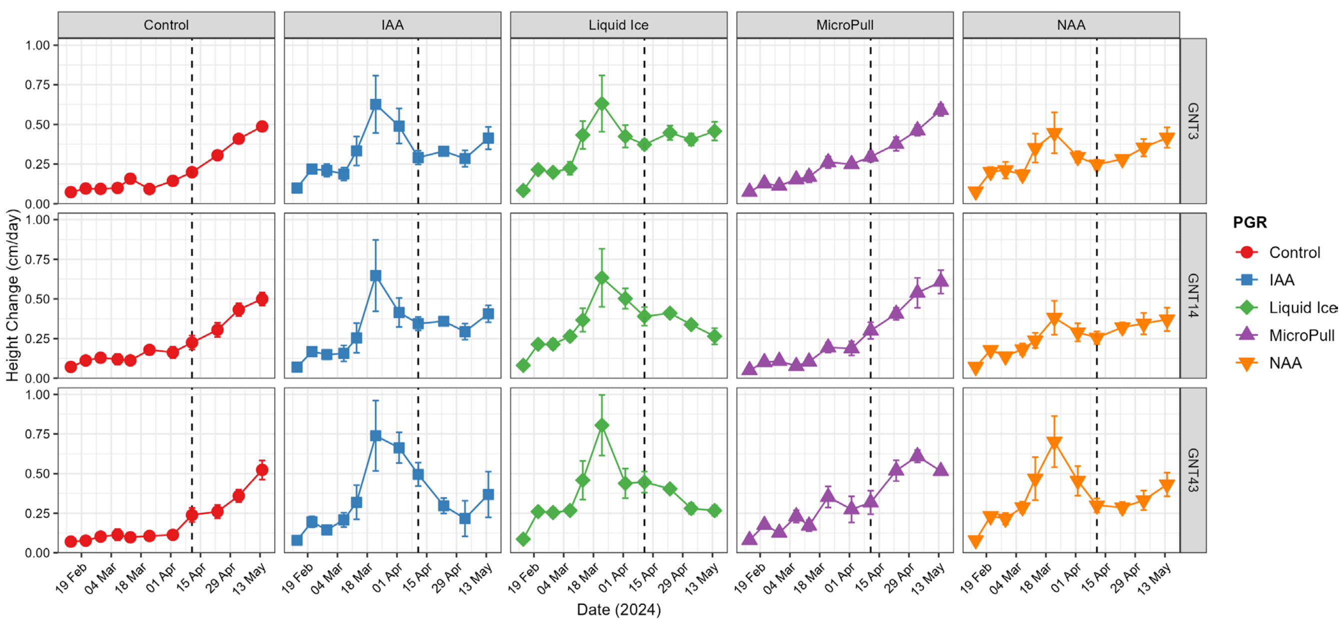

FIGURE 3 | Estimated daily height change profiles for Miscanthus plug seedlings. Line plots of mean daily height increment (cm day^{-1}), faceted by hybrid (rows) and PGR treatment (columns). Symbols indicate mean ± SE ($n = 6$). The vertical dashed line indicates the final PGR application date.

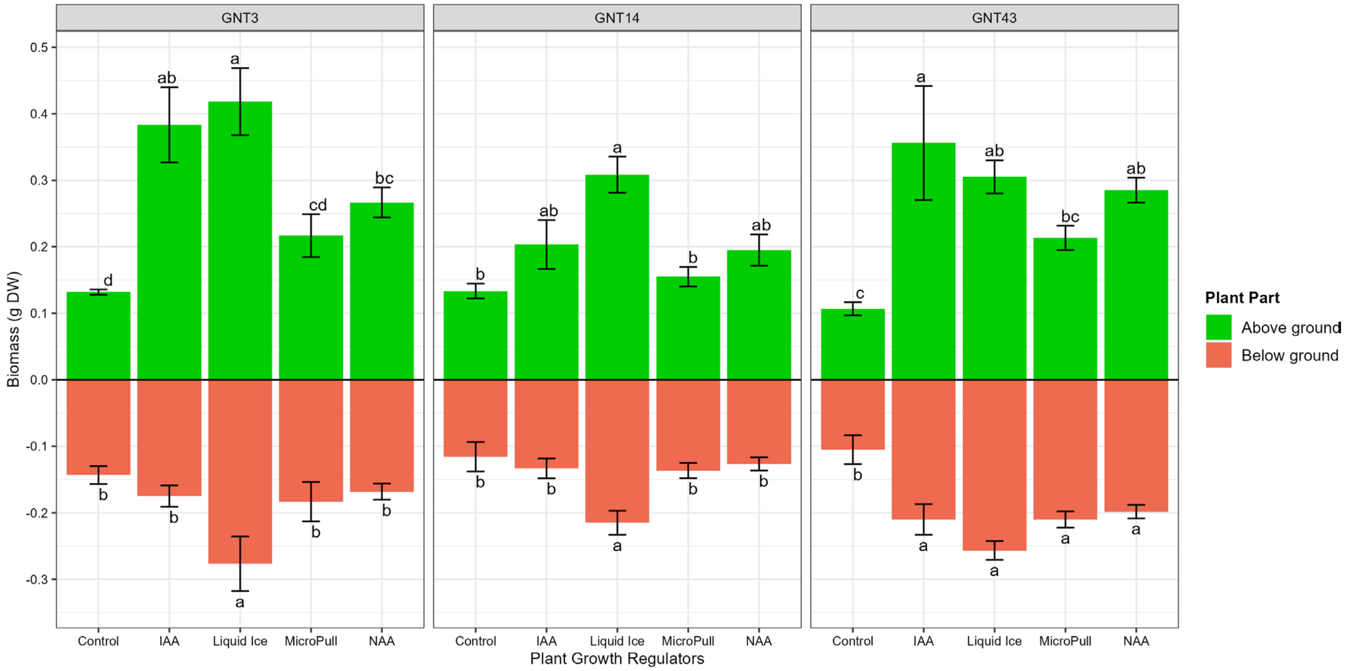

FIGURE 4 | Biomass production (g DW) of Miscanthus hybrids (GNT14, GNT3, and GNT43) subjected to different plant growth regulator (PGR) treatments. Coloured bars represent above-ground biomass (leaves and stems; green) and below-ground biomass (roots; brown) ± SE ($n = 6$). Different letters above error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

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.

3.4 | 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 PGRs, 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.

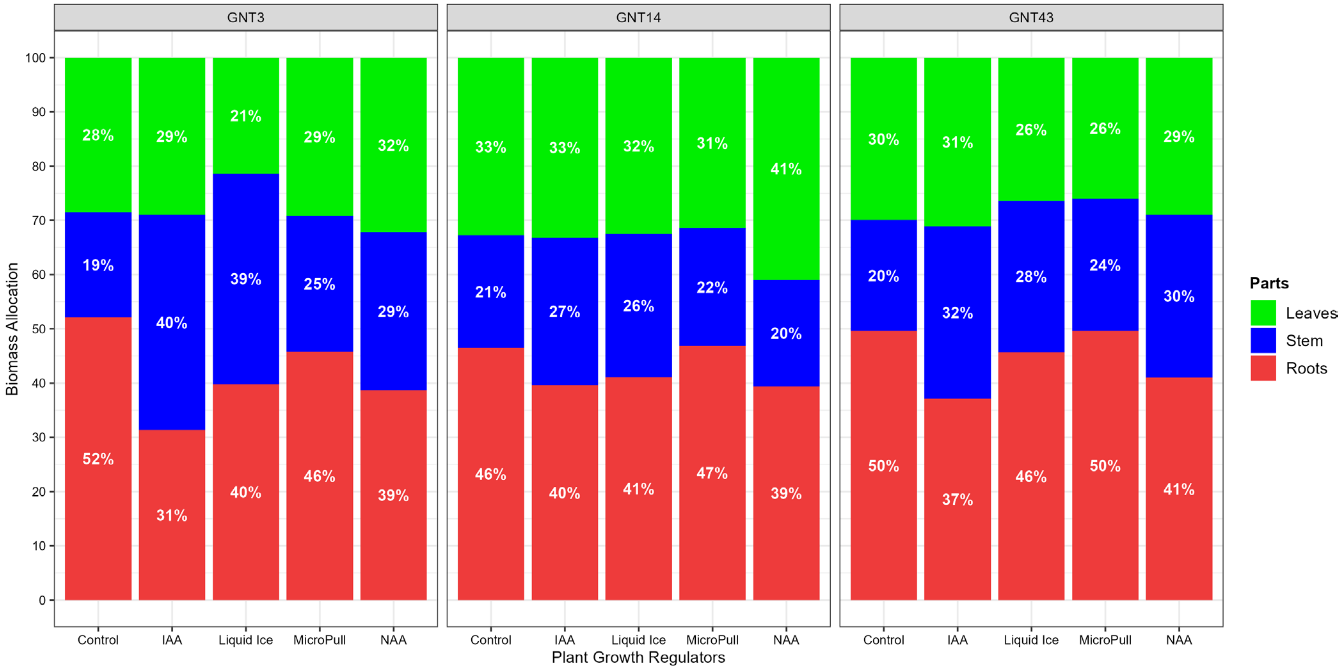

FIGURE 5 | Dry biomass allocation patterns in Miscanthus hybrids (GNT14, GNT3, and GNT43) under various PGR treatments. Bars indicate mean biomass distribution (%) among plant components (roots, stems, leaves) for each treatment within hybrids. Percentages reflect the proportional allocation averaged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

3.5 | 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.

3.6 | 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.

3.7 | 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 girth 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.

3.8 | 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.

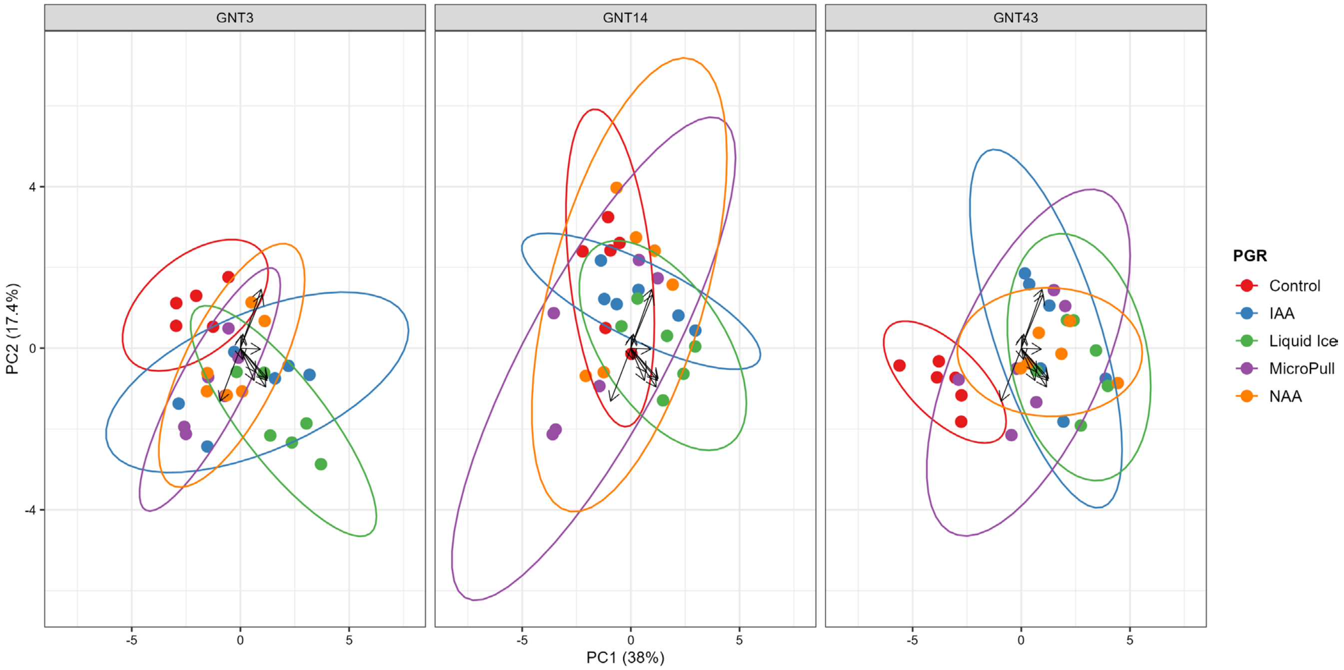

FIGURE 6 | Principal component analysis (PCA) of morphological, biomass, and photosynthetic traits for Miscanthus hybrids under plant growth regulator treatments. Points represent individual replicates; ellipses indicate confidence intervals. Trait loadings are shown as vectors (A, ik; B, etr_max; C, 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).

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.

3.9 | 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.

4 | 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.

4.1 | Germination and Early Establishment

MicroPull had been observed to delay germination in GNT14 and reduce 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 and Abd El-Wahed 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 a different dosage is required to command any effect.

4.2 | 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 PGRs 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).

4.3 | 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 (Figures 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 and Oliveira 2024).

4.4 | 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.

4.5 | 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 PGRs 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 and Munné-Bosch 2021). Understanding the mechanistic basis for these hybrid-specific photosynthetic shifts could inform more nuanced deployment of PGRs in plug propagation.

4.6 | 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, the degree of separation among treatments was generally modest and accompanied by substantial overlap. This suggests that while PGRs 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 and 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, Wilson, et al. 2023).

4.7 | 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 a 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, Awty-Carroll, et al. (2023) 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 optimising *Miscanthus* plug production. By modulating traits like stem 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, Awty-Carroll, et al. (2023) 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 (PGR) applications with complementary plug optimisation strategies, for example, 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.

5 | 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 aboveground 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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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

Ashman, C., D. Awty-Carroll, M. Mos, P. Robson, and J. Clifton-Brown. 2018. "Assessing Seed Priming, Sowing Date, and Mulch Film to Improve the Germination and Survival of Direct-Sown *Miscanthus sinensis* in the United Kingdom." *Global Change Biology. Bioenergy* 10, no. 9: 612–627. https://doi.org/10.1111/gcbb.12518.

Ashman, C., D. Awty-Carroll, R. L. Rowe, and J. Clifton-Brown. 2023. "Development of a Miscanthus Plug Planting Protocol Using Mulch Film to Ensure Establishment and Yield From Seed." *Global Change Biology. Bioenergy* 15, no. 6: 534–548. https://doi.org/10.1111/gcbb.13044.

Ashman, C., R. Wilson, M. Mos, J. Clifton-Brown, and P. Robson. 2023. "Improving Field Establishment and Yield in Seed Propagated Miscanthus Through Manipulating Plug Size, Sowing Date and Seedling Age." *Frontiers in Plant Science* 14: 1095838. https://doi.org/10.3389/fpls.2023.1095838.

Baker, N. R. 2008. "Chlorophyll Fluorescence: A Probe of Photosynthesis In Vivo." *Annual Review of Plant Biology* 59: 89–113. https://doi.org/10.1146/annurev.arplant.59.032607.092759.

Clifton-Brown, J., J. Breuer, and M. B. Jones. 2007. "Carbon Mitigation by the Energy Crop, Miscanthus." *Global Change Biology* 13, no. 11: 2296–2307. https://doi.org/10.1111/j.1365-2486.2007.01438.x.

Clifton-Brown, J., A. Harfouche, M. D. Casler, et al. 2019. "Breeding Progress and Preparedness for Mass-Scale Deployment of Perennial Lignocellulosic Biomass Crops Switchgrass, Miscanthus, Willow and Poplar." *Global Change Biology. Bioenergy* 11, no. 1: 118–151. https://doi.org/10.1111/gcbb.12566.

Clifton-Brown, J., A. Hastings, M. Mos, et al. 2017. "Progress in Upscaling Miscanthus Biomass Production for the European Bio-Economy With Seed-Based Hybrids." *Global Change Biology. Bioenergy* 9, no. 1: 6–17. https://doi.org/10.1111/gcbb.12357.

Cristofano, F., C. El-Nakhel, and Y. Rouphael. 2021. "Biostimulant Substances for Sustainable Agriculture: Origin, Operating Mechanisms and Effects on Cucurbits, Leafy Greens, and Nightshade Vegetables Species." *Biomolecules* 11, no. 8: 1103. https://doi.org/10.3390/biom11081103.

Dang, L. V., S. Matsuura, R. Wagai, et al. 2025. "Quantifying the Dynamics of Miscanthus × Giganteus Belowground Biomass and Soil Carbon and Nitrogen Stocks for Three Years of Cultivation on Andosols, Iwate, Japan." *Soil Science and Plant Nutrition* 71: 1–11. https://doi.org/10.1080/00380768.2025.2495701.

Dewi, R. K., M. Fukuda, N. Takashima, A. Yagioka, and M. Komatsuzaki. 2022. "Soil Carbon Sequestration and Soil Quality Change Between no-Tillage and Conventional Tillage Soil Management After 3 and 11 Years of Organic Farming." *Soil Science and Plant Nutrition* 68, no. 1: 133–148. https://doi.org/10.1080/00380768.2021.1997552.

Duong, M. V., J. W. Chung, V. G. Ha, H. Moon, J. K. Yu, and Y. S. So. 2024. "Prohexadione-Calcium Mitigates the Overgrowth of Corn Seedlings." *Agronomy* 14, no. 2: 371. https://doi.org/10.3390/agronomy14020371.

Ellouzi, H., I. Ben Slimene Debez, S. Amraoui, et al. 2024. "Effect of Seed Priming With Auxin on ROS Detoxification and Carbohydrate Metabolism and Their Relationship With Germination and Early Seedling Establishment in Salt Stressed Maize." *BMC Plant Biology* 24: 704. https://doi.org/10.1186/s12870-024-05413-w.

El-Mergawi, R. A., and M. S. A. Abd El-Wahed. 2020. "Effect of Exogenous Salicylic Acid or Indole Acetic Acid on Their Endogenous Levels, Germination, and Growth in Maize." *Bulletin of the National Research Centre* 44: 167. https://doi.org/10.1186/s42269-020-00416-7.

Fei, H., M. Crouse, Y. A. Papadopoulos, and J. K. Vessey. 2020. "Improving Biomass Yield of Giant Miscanthus by Application of Beneficial Soil Microbes and a Plant Biostimulant." *Canadian Journal of Plant Science* 100, no. 1: 29–39. https://doi.org/10.1139/cjps-2019-0012.

Fox, J., and S. Weisberg. 2019. *An R Companion to Applied Regression*. 3rd ed. Sage Publications. https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

Gautrat, P., S. E. Matton, L. Oskam, and S. S. Shetty. 2025. "Lights, Location, Action: Shade Avoidance Signalling Over Spatial Scales." *Journal of Experimental Botany* 76, no. 3: 695–711. https://doi.org/10.1093/jxb/erae217.

Gruntman, M., D. Groß, M. Májeková, and K. Tielbörger. 2017. "Decision-Making in Plants Under Competition." *Nature Communications* 8, no. 1: 2235. https://doi.org/10.1038/s41467-017-02147-2.

Hastings, A., J. Clifton-Brown, M. Wattenbach, C. P. Mitchell, P. Stampfl, and P. Smith. 2009. "Future Energy Potential of Miscanthus in Europe." *GCB Bioenergy* 1, no. 2: 180–196.

Hastings, A., M. Mos, J. A. Yesufu, et al. 2017. "Economic and Environmental Assessment of Seed and Rhizome Propagated Miscanthus in the UK." *Frontiers in Plant Science* 8: 1058. https://doi.org/10.3389/fpls.2017.01058.

Heaton, E. A., F. G. Dohleman, and S. P. Long. 2008. "Meeting US Biofuel Goals With Less Land: The Potential of Miscanthus." *Global Change Biology* 14: 2000–2014. https://doi.org/10.1111/j.1365-2486.2008.01662.x.

Herdean, A. 2022. "PSI FluorCAM Protocol for Rapid Light Curve With Far-Red Pre-Illumination (Version 1) Protocols.io." https://doi.org/10.17504/protocols.io.x54v9deypg3e/v1.

Jolliffe, I. T., and J. Cadima. 2016. "Principal Component Analysis: A Review and Recent Developments." *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* 374, no. 2065: 20150202. https://doi.org/10.1098/rsta.2015.0202.

Katelevskij, V., M. Gumentyk, and M. Kharytonov. 2020. "Plant Growth Stimulants Influence on Miscanthus × Giganteus Biomass Indexes in Forest-Steppe Zone of Ukraine." *Scientific Papers. Series A. Agronomy* 63, no. 1: 341–345.

Kaushal, P., N. Ali, S. Saini, P. K. Pati, and A. M. Pati. 2023. "Physiological and Molecular Insight of Microbial Biostimulants for Sustainable Agriculture." *Frontiers in Plant Science* 14: 1041413. https://doi.org/10.3389/fpls.2023.1041413.

Lavania, D., N. M. Linh, and E. Scarpella. 2021. "Of Cells, Strands, and Networks: Auxin and the Patterned Formation of the Vascular System." *Cold Spring Harbor Perspectives in Biology* 13, no. 6: a039958. https://doi.org/10.1101/cshperspect.a039958.

Lee, M.-S., R. A. Boyd, and D. R. Ort. 2021. "The Photosynthetic Response of C3 and C4 Bioenergy Grass Species to Fluctuating Light." *GCB Bioenergy* 14: 37–53. https://doi.org/10.1111/gcbb.12899.

Lenth, R. V. 2023. "EMMEANS: Estimated Marginal Means, aka Least-Squares Means. R package version 1.10.0." https://CRAN.R-project.org/package=emmeans.

Lewandowski, I., J. Clifton-Brown, L. M. Trindade, et al. 2016. "Progress on Optimizing Miscanthus Biomass Production for the European Bioeconomy: Results of the EU FP7 Project OPTIMISC." *Frontiers in Plant Science* 7: 1620. https://doi.org/10.3389/fpls.2016.01620.

Li, X., Q. Zhong, Y. Li, et al. 2016. "Triacontanol Reduces Transplanting Shock in Machine-Transplanted Rice by Improving the Growth and Antioxidant Systems." *Frontiers in Plant Science* 7: 872. https://doi.org/10.3389/fpls.2016.00872.

Müller, M., and S. Munné-Bosch. 2021. "Hormonal Impact on Photosynthesis and Photoprotection in Plants." *Plant Physiology* 185, no. 4: 1500–1522. https://doi.org/10.1093/plphys/kiaa119.

Murchie, E. H., and T. Lawson. 2013. "Chlorophyll Fluorescence Analysis: A Guide to Good Practice and Understanding Some New Applications." *Journal of Experimental Botany* 64: 3983–3998. https://doi.org/10.1093/jxb/ert208.

Nunn, C., A. F. Hastings, O. Kalinina, et al. 2017. "Environmental Influences on the Growing Season Duration and Ripening of Diverse Miscanthus Germplasm Grown in Six Countries." *Frontiers in Plant Science* 8: 907. https://doi.org/10.3389/fpls.2017.00907.

Pidlisnyuk, V., T. Stefanovska, O. Zhukov, et al. 2022. "Impact of Plant Growth Regulators to Development of the Second Generation Energy Crop Miscanthus × Giganteus Produced Two Years in Marginal Post-Military Soil." *Applied Sciences* 12, no. 2: 881. https://doi.org/10.3390/app12020881.

Pietrini, F., M. A. Iannelli, and A. Massacci. 2011. "Anthocyanin Accumulation in the Leaves of *Phragmites australis* in Response to High Light: Flavonoid Biosynthesis and Photoprotection." *Plant, Cell & Environment* 24, no. 9: 1051–1059. https://doi.org/10.1046/j.1365-3040.2001.00764.x.

Poorter, H., K. J. Niklas, P. B. Reich, J. Oleksyn, P. Poot, and L. Mommer. 2012. "Biomass Allocation to Leaves, Stems and Roots: Meta-Analysis of Interspecific Variation and Environmental Control." *New Phytologist* 193, no. 1: 30–50. https://doi.org/10.1111/j.1469-8137.2011.03952.x.

R Core Team. 2024. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.R-project.org/.

Ralph, P. J., and R. Gademann. 2005. "Rapid Light Curves: A Powerful Tool to Assess Photosynthetic Activity." *Aquatic Botany* 82, no. 3: 222–237. https://doi.org/10.1016/j.aquabot.2005.02.006.

Reich, P. B. 2014. "The World-Wide "Fast–Slow" Plant Economics Spectrum: A Traits Manifesto." *Proceedings of the National Academy of Sciences* 111, no. 8: 11533–11538. https://doi.org/10.1073/pnas.1419991111.

Severino, L. S., and E. Oliveira. 2024. "Plant Growth Regulators Influence the Height and Biomass Partition of Castor Plants." *Revista Brasileira de Engenharia Agrícola E Ambiental* 28, no. 5: e276554. https://doi.org/10.1590/1807-1929/agriambi.v28n5e276554.

Shavyrkina, N. A., V. V. Budaeva, E. A. Skiba, Y. A. Gismatulina, and G. V. Sakovich. 2023. "Review of Current Prospects for Using Miscanthus-Based Polymers." *Polymers* 15, no. 14: 3097. https://doi.org/10.3390/polym15143097.

Szpunar-Krok, E., M. Staniak, and S. Kocira. 2024. "Physiological Response of *Miscanthus sinensis* to Biostimulant Application Under Salt Stress." *Agriculture* 14, no. 1: 33. https://doi.org/10.3390/agriculture14010033.

Tang, J., Z. Zhang, S. A. Tung, B. Lu, and W. Yang. 2024. "Plant Growth Regulators Improve Root Growth of Rice Seedlings After Mechanical Transplanting and Increase Grain Yield." *Experimental Agriculture* 60: e8. https://doi.org/10.1017/S0014479724000048.

Urbutis, M., K. Laužikė, and G. Samuolienė. 2023. "Exogenous Phytohormones: Effects on Lettuce Photosynthesis." *Antioxidant Response and Growth. Horticulturae* 9, no. 7: 792. https://doi.org/10.3390/horticulturae9070792.

Vercruyssen, L., V. B. Tognetti, N. Gonzalez, et al. 2015. "GROWTH REGULATING FACTOR5 Stimulates Arabidopsis Chloroplast Division, Photosynthesis, and Leaf Longevity." *Plant Physiology* 167, no. 3: 817–832. https://doi.org/10.1104/pp.114.256180.

Williamson, M. M., G. W. T. Wilson, and D. C. Hartnett. 2012. "Controls on Bud Activation and Tiller Initiation in C3 and C4 Tallgrass Prairie Grasses: The Role of Light and Nitrogen." *Botany* 90, no. 12: 1221–1228. https://doi.org/10.1139/b2012-091.

Winkler, B., A. Mangold, M. von Cossel, et al. 2020. "Implementing Miscanthus Into Farming Systems: A Review of Agronomic Practices, Capital and Labour Demand." *Renewable and Sustainable Energy Reviews* 132: 110053. https://doi.org/10.1016/j.rser.2020.110053.

Wu, P., C. Ashman, D. Awty-Carroll, P. Robson, and J. Clifton-Brown. 2021. "Optimizing Seed-Based Miscanthus Plug Plant Production With Supplemental Heat and Light, Compost Type and Volume." *Global Change Biology. Bioenergy* 14: 624–638. https://doi.org/10.1111/gcbb.12920.

Zhao, Y., T. Wang, W. Zhang, M. Wang, and T. Li. 2020. "IAA Priming Improves the Germination and Seedling Growth in Cotton (*Gossypium hirsutum* L.) Under Salt Stress." *Industrial Crops and Products* 145: 111990.

Zheng, C., R. L. Rowe, and I. S. Donnison. 2021. "Two-Step Seed-Derived Plug Propagation System for Miscanthus Improves Establishment and Biomass Yield in Field Trials." *Global Change Biology. Bioenergy* 13, no. 6: 977–991. https://doi.org/10.1111/gcbb.12820.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2010. *Mixed Effects Models and Extensions in Ecology With R*. Springer. https://doi.org/10.1007/978-0-387-87458-6.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Summary of factorial ANOVA p-values for treatment (PGR), hybrid, and their interaction across all measured morphological, biomass, and photosynthetic traits. **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.