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Research Article

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Co-formulated plant growth regulators applied before the juvenile-to-adult transition prime microRNA-mediated stress defence and improve yield in sorghum (Sorghum bicolor L. Moench)

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Abstract

Sorghum (Sorghum bicolor L. Moench) yield improvement has stagnated despite the crop's inherent advantages as a C4 species adapted to water-limited environments. In Australia, yield gaps between water-limited potential and attainable on-farm yields average 59% of potential, representing a major unrealised opportunity (Garba et al., 2025). Biotic and abiotic stress remain primary yield limiters, and existing genetic and management tools have failed to close this gap. This study reports findings from eleven replicated field trials conducted at multiple experimental sites across three production regions of South-East Queensland, Australia, over seven seasons (2014-15 to 2020-21), evaluating the response of commercial grain sorghum to co-formulated plant growth regulators (PGRs) -- comprising a gibberellin biosynthesis inhibitor and an ethylene precursor -- applied at the 2-4 leaf growth stage so that the absorbed phytohormones could become physiologically active as plants entered the fourth- to fifth-leaf juvenile-to-adult (JA) phase transition. The central hypothesis was that exogenous PGRs applied during this early developmental window interact with endogenous microRNA-mediated signalling pathways -- particularly miR156 and miR172 -- to prime the plant's own defence and resource allocation systems for the remainder of the growing season. Across six commercial varieties and highly variable seasonal conditions including drought years, ANOVA confirmed statistically significant treatment effects on yield or associated agronomic outcomes across the trial programme. Yield responses ranged from 107% to 134% of the untreated control in responsive trials, with T3 (medium-low rate) the most consistently effective treatment across seasons, experimental sites, and varieties. Under severe drought, treated plants showed significantly reduced grain screenings (from 27% to 14%) and enhanced staygreen, consistent with a shift from growth optimisation to stress defence. Broad-spectrum disease suppression was recorded across multiple pathogens -- consistent with activation of systemic acquired resistance pathways mediated by ethylene and jasmonic acid signalling. The variety independence of responses, the adaptive switching between growth and defence modes across seasons, and the timing specificity of the early application window collectively support the proposed priming mechanism. These findings contribute a management dimension to the G×E×M framework needed to break the established sorghum yield barrier, offering yield improvements equivalent to approximately 20 years of conventional genetic progress through a single, scalable, variety-independent intervention.

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1. Introduction

1.1 The Sorghum Yield Plateau and the Case for Management Innovation

Sorghum (Sorghum bicolor L. Moench) is the fifth most important cereal crop globally, providing nutrition for over 500 million people across arid and semi-arid regions where few other crops can reliably produce grain (FAO, 1999; Vara & Staggenborg, 2010). Its C4 photosynthetic pathway confers superior water and nitrogen use efficiency relative to C3 cereals, making it a crop of increasing strategic importance as climate volatility intensifies and arable land becomes more constrained (Mundia et al., 2019; Rakshit et al., 2014).

Despite these inherent advantages, sorghum yield improvement has stagnated. While maize and wheat achieved dramatic yield gains through the Green Revolution -- driven by the introduction of semi-dwarfing genes that improved harvest index and permitted intensified management -- sorghum has not followed the same trajectory. Long-term average yield increases in Australian sorghum are approximately 1% per annum, far below the 2.4% annual increase estimated as necessary to meet projected global food demand by 2050 (Hammer, 2011; Narayanan et al., 2013; Ray et al., 2013). Recent modelling of Australian sorghum production systems confirms that yield gaps between water-limited potential and attainable on-farm yields average 59% of potential, with nitrogen management and sowing date identified as primary drivers of this gap (Garba et al., 2025). Simulation-based crop design optimisation using the G×E×M framework has further demonstrated that maturity, tillering and planting density combinations can be optimised to simultaneously improve yield and reduce production risk -- but that management remains an underutilised lever in current production systems (Durrington et al., 2025).

The reasons for this divergence are partly genetic. Dwarfing genes introduced into sorghum to enable mechanical harvesting -- particularly dw3 -- reduce not only plant height but also shoot biomass, radiation capture, tiller number and panicle size, ultimately constraining the source-sink relationships that underpin yield (George-Jaeggli et al., 2011, 2013). Unlike wheat, where semi-dwarfing genes (Rht-B1, Rht-D1) improved harvest index by limiting gibberellin-mediated stem extension while preserving reproductive capacity, sorghum dwarfing operates through auxin transport disruption, with pleiotropic consequences for overall plant architecture and productivity (Hilley et al., 2016; Salamini, 2003).

Hammer (2011) proposed that breaking the sorghum yield barrier requires moving beyond the traditional genetic (G) × environment (E) framework to incorporate management (M) as an active variable -- a G×E×M approach. This study addresses that gap directly. The question driving this research was whether exogenous plant growth regulators (PGRs), applied at a precisely defined developmental window, could activate endogenous biological mechanisms capable of improving yield stability and stress resilience across variable seasons, sites and varieties -- without additional land, water or fertiliser inputs.

1.2 Plant Growth Regulators as Phytohormone Signals

Plant growth regulators (PGRs) are natural or synthetic compounds that, when applied exogenously at low concentrations, interact with endogenous phytohormone pathways to alter

plant growth, development, and stress responses (Rademacher, 2000, 2015). Commercial PGR formulations broadly fall into two functional classes: gibberellin (GA) biosynthesis inhibitors, which limit cell elongation and redirect assimilate allocation; and ethylene (ET) precursors, which modulate a wide range of developmental and defence-related processes (Albacete et al., 2014; Rademacher, 2015).

Critically, PGRs do not act as simple growth suppressants. Their effects are mediated through complex phytohormone crosstalk involving gibberellins, ethylene, cytokinins, auxins, abscisic acid (ABA), salicylic acid (SA) and jasmonic acid (JA) -- the same hormonal network that regulates plant responses to biotic and abiotic stress (Denance et al., 2013; Derksen et al., 2013). Ethylene in particular has emerged as a central integrative node in this network, coordinating the redistribution of metabolic resources between growth and defence through the ETR-CTR1-EIN2-EIN3 signalling module, with downstream crosstalk involving JA, SA, ABA, auxin and GA enabling fine-tuned coordination of growth inhibition, immune activation and stress acclimation (Mia et al., 2026). Importantly, auxin itself acts not as a direct controller of specific cell types but as a facilitator that associates with tissue-specific transcription factors to regulate developmental outputs -- a principle demonstrated across land plant lineages (Flores-Sandoval & Bowman, 2025). This facilitatory role of auxin is directly relevant to the proposed mechanism: PGR-mediated modulation of auxin and gibberellin signalling during the JA transition may amplify pre-existing regulatory networks rather than imposing entirely new developmental programmes.

The outcome of PGR application is therefore not fixed but context-dependent: in favourable seasons, altered hormone balance may optimise canopy architecture and sink-source relations; under stress, the same hormonal shifts may activate defence pathways that limit pathogen damage or conserve water. This dual capacity -- growth optimisation under favourable conditions, stress mitigation under adverse ones -- is precisely what was observed across the multi-year, multi-site field programme reported here.

1.3 The Juvenile-to-Adult Transition as a Critical Developmental Window

In sorghum and maize, the juvenile-to-adult (JA) phase transition is a fundamental developmental checkpoint that determines the extent of vegetative growth before the plant commits to reproductive development. This transition involves coordinated morphological and genetic changes regulated by two conserved microRNA markers -- miR156 and miR172 -- that establish lifelong patterns of vegetative architecture, reproductive capacity, and stress responsiveness.

In sorghum specifically, Hashimoto et al. (2019) demonstrated that miR156 expression is highest in the second to fourth leaves and then gradually declines, while miR172 expression follows the opposite pattern. Morphological changes in leaf size, leaf shape, trichome density and shoot apical meristem size confirm that the second and third leaves represent the juvenile phase, the fourth and fifth leaves are in the transition stage, and the sixth and upper leaves are in the adult phase. The JA phase transition therefore occurs during the fourth and fifth leaf stages in sorghum. PGR application in this study was targeted earlier, at the 2-4 leaf growth stage, so that the absorbed phytohormones could become physiologically active as plants entered the transition.

The molecular mechanism underpinning this transition is well characterised. miR156 suppresses SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) transcription factors during the juvenile phase, maintaining vegetative identity; its gradual decline allows miR172 to accumulate, promoting the transition to adult phase and reproductive competence (Osadchuk et al., 2021). In maize, leaf-derived signals -- including hormones, stress signals, and sugars -- attenuate miR156 expression and thus regulate the duration of the juvenile phase through epigenetic remodelling (Osadchuk et al., 2021). In wheat, miR156 and its targeted SPL genes interact directly with the gibberellin pathway: DELLA proteins, negative regulators of GA signalling, physically interact with SPL proteins to modulate their transcriptional activity, creating a regulatory feedback loop that links the age pathway to hormonal status (Liu et al., 2026). In cereals more broadly, the miR156-SPL network has been shown to control spikelet fate and meristem activity, with miR156 levels

elevated under stress conditions -- including nitrogen deficiency and heat -- potentially halting reproductive development and contributing to yield losses (Alqudah et al., 2026).

Critically for the present study, miR156 has been identified as a central regulator of plant stress adaptation in sorghum itself. High-throughput sequencing of small RNA libraries from drought-tolerant and drought-susceptible sorghum genotypes revealed that miR156 is differentially regulated under drought stress, with expression patterns linked to genotype-specific stress tolerance mechanisms (Katiyar et al., 2015). The miR156-SPL module directly regulates ethylene production pathways in multiple species (Li et al., 2024), establishing a mechanistic link between the developmental transition and the hormonal defence network. Furthermore, miR156 targets include transcription factors from the SPL, WRKY and ARF families -- the same families implicated in plant immune responses and stress signalling (Katiyar et al., 2015; Jeyakumar et al., 2020).

The hypothesis tested in this study was that exogenous PGR application before the JA transition phase -- by modulating gibberellin and ethylene signalling so that the absorbed phytohormones became active during the transition -- could amplify or initiate the endogenous miRNA-mediated programming that governs plant defence and resource allocation for the remainder of the crop's lifecycle. If correct, this would represent a management-accessible mechanism for improving sorghum yield stability that is variety-independent, scalable, and achievable without additional resource inputs.

1.4 PGRs in Cereal Production: Established Use and the C4 Gap

The use of PGRs in C3 cereal production -- particularly wheat and barley -- is well established. In Europe, an estimated 70% of cereal crops receive PGR treatment, primarily to reduce lodging risk by limiting gibberellin-mediated stem elongation (Rademacher, 2015). Beyond lodging control, PGR application in cereals has been associated with improved root architecture, enhanced nutrient, and water use efficiency, and in some cases improved disease resistance (Berry et al., 2004; Rademacher, 2015). Recent work in maize has demonstrated that ethephon application improves lodging resistance through increased lignin biosynthesis -- upregulating key enzymes including cinnamyl alcohol dehydrogenase (CAD), phenylalanine ammonia-lyase (PAL) and 4-coumarate-CoA ligase (4CL) -- with yield benefits dependent on application timing and rate (Liu et al., 2025; Zhang et al., 2024).

However, the direct translation of C3 cereal PGR protocols to C4 crops such as sorghum is not straightforward. Height control in wheat operates through gibberellin signalling pathways involving DELLA proteins, whereas in sorghum and maize, height is regulated primarily through auxin transport mechanisms (Hilley et al., 2016; Salamini, 2003). This fundamental difference in the hormonal architecture of height control means that application timing, product selection and expected responses differ substantially between species. Published research on exogenous PGR application to grain sorghum was extremely limited at the commencement of this study. The work reported here represents, to our knowledge, the most extensive replicated field investigation of PGR effects on grain sorghum conducted to date.

1.5 Aims of This Study

This study aimed to:

- Determine whether co-formulated PGRs applied at the 2-4 leaf growth stage, before the juvenile-to-adult transition in sorghum, could improve yield across variable seasons, experimental sites, production regions and commercial varieties.
- Characterise the nature of plant responses -- distinguishing between growth-optimising effects in favourable seasons and stress-mitigating effects under biotic and abiotic pressure.

- Provide field evidence consistent with the hypothesis that early PGR application primes endogenous miRNA-mediated defence pathways during the JA transition phase.
- Establish a management framework for PGR use in sorghum that contributes to the G×E×M approach needed to break the established yield barrier.

2. Materials and Methods

2.1 Production Regions, Experimental Sites and Seasons

Eleven analytical trials were conducted over seven seasons (2014-15 to 2020-21) at multiple experimental sites within three production regions of South-East Queensland, Australia: the Felton Valley on the Darling Downs, the Lockyer Valley around Gatton and Forest Hill, and the Fassifern Valley around Kalbar. An experimental site refers to the individual paddock or trial location used in a given season; several distinct sites were used within each broader region. The programme spanned favourable growing years (2015, 2016, 2018, 2021), moderate drought (2017) and severe drought (2019, 2020).

Felton Valley region, Darling Downs (representative coordinates 27°47'51"S, 151°46'25"E; elevation approximately 400 m). Subtropical climate with summer temperatures of 28–34°C and annual rainfall of 600–1,000 mm. Soils are vertosols (brown-grey to black) with high fertility and substantial water-holding capacity. Trials were conducted from 2015 to 2019 at multiple experimental paddock sites across the wider Felton Valley area.

Lockyer Valley region — Gatton and Forest Hill. Experimental sites included the University of Queensland Research Farm, Gatton (27.5611°S, 152.2755°E; elevation 94 m), and a separate commercial-field site near Forest Hill. The region has a humid subtropical climate, summer-dominant rainfall and high-fertility black soils. Trials were conducted at two distinct experimental sites within the region in 2018 and 2020.

Fassifern Valley region — Kalbar. Two separate experimental sites, approximately 30 km apart, were used in the Kalbar area in 2021. Representative coordinates for the region are 27.8109°S, 152.6651°E; elevation approximately 90 m. The region has a subtropical climate, average annual rainfall of approximately 900 mm with summer dominance, summer temperatures of 28–44°C, and flat, well-drained, high-fertility alluvial soils (pH approximately 7).

2.2 Experimental Design

Experiments were established as Randomised Block Designs (RBD) within commercial sorghum crops, using the grower's variety, fertiliser programme, seeding rate and planting equipment. This approach was deliberately chosen to maximise agronomic realism and scalability of findings.

From 2015 to 2019, seven PGR treatment levels plus an untreated control were evaluated, replicated four times per trial. In 2020 and 2021, treatment number was reduced to three levels — those that had demonstrated the most consistent positive responses in earlier trials — replicated four times.

Plot dimensions were 2 m wide × 10 m long, with untreated buffer rows on each side. Assessment rows were determined by row spacing: for 1 m spacing, both rows were assessed; for 75 cm spacing, the middle row of three treated rows; for 50 cm spacing, the two middle rows of four treated rows.

2.3 Genotypes

Six commercially available hybrid sorghum varieties were evaluated across the trial programme: G33, G44, G66, MR43, Mr Buster and Tiger. Varieties were selected by the host grower based on local adaptation and commercial practice. The inclusion of multiple varieties across multiple

seasons was deliberate, to test whether PGR responses were variety-specific or broadly applicable across commercial germplasm.

2.4 PGR Treatments and Application

Treatments consisted of co-formulated mixtures of a gibberellin biosynthesis inhibitor and an ethylene precursor, applied with a canola oil ester adjuvant to improve foliar uptake and rainfastness. Seven treatment levels were evaluated, representing a dose-response series from low to high co-formulation concentration, plus an untreated control. Product identities and specific application rates are not reported here, as the focus of this study is on the biological responses to PGR class and timing rather than product-specific recommendations.

Application timing was the critical variable. Treatment was applied before the juvenile-to-adult transition, from the point at which approximately 70% of plants had fully extended their third leaf through to the late fourth-leaf growth stage, so that the absorbed phytohormones could become physiologically active as plants entered the fourth- to fifth-leaf transition. Growth stage was identified by counting leaf collars on the plant stem. Preliminary exploratory trials (2012–2014, data not presented) identified this early application period as the window that consistently produced positive responses; application at the analogous stage used in wheat (equivalent to the 6–7 leaf stage in sorghum) produced negative outcomes including stunted plants, compact panicles and increased disease susceptibility.

Applications were made using a manual diaphragm pump knapsack sprayer fitted with a 2 m boom carrying five air-induction nozzles at 50 cm spacing, operated at 2.5 bar pressure and 5 km/hr walking speed. Boom height was maintained at 50 cm above the crop canopy. All summer trials were sprayed between 05:00 and 09:00 to minimise temperature, evaporation and wind effects. Personal protective equipment was worn throughout.

2.5 Measurements and Observations

The following parameters were assessed at each trial:

- Grain yield (kg/m or kg/plot): assessed at physiological maturity by mechanical small-plot harvester or hand-harvested 1–2 m row sections, processed and weighed with digital scales.
- Crop height (cm): measured manually from sample plants within the assessment area.
- Panicle number (panicles per plot): counted manually.
- Tiller abortion (aborted tillers per plot): counted manually.
- Disease score: visual assessment on a 1–10 scale (1 = no disease, 10 = severe infection). Pathogens identified included Fusarium stalk rot (Fusarium spp.), tar spot (Phyllachora sacchari), rust (Puccinia purpurea), anthracnose (Colletotrichum graminicola) and grain mould.
- Staygreen score: visual assessment of canopy greenness retention post-anthesis (1–5 scale).
- Grain screenings (%): proportion of grain below standard screen size, assessed using an Agtator at The Leslie Research Centre, Toowoomba.
- Grain colour: visual assessment where relevant.

Photographic records were maintained throughout the trial programme to document morphological responses including root architecture, canopy structure, disease expression and grain quality.

2.6 Statistical Analysis

Data were analysed using Analysis of Variance (ANOVA) to test for significant effects of treatment and, where applicable, genotype and genotype × treatment interaction. Where significant effects were detected, post-hoc Tukey Honest Significant Difference (HSD) tests were applied to identify

treatment differences, with correction for multiple comparisons. All analyses were conducted in R, using the emmeans package for Tukey HSD tests and multcomp for compact letter displays. The significance threshold was set at α = 0.05 throughout. Statistical analysis was conducted by Dr Vincent Mellor, Senior Biometrics Consultant, School of Agriculture and Food Sciences, The University of Queensland.

Results approaching but not reaching the 0.05 significance threshold are reported where the direction and magnitude of response are consistent with the broader pattern of results and contribute to interpretation of the biological mechanisms proposed.

3. Results

3.1 Overview of Seasonal Conditions and Trial Outcomes

Across the eleven analytical trials, seasonal conditions ranged from highly favourable (2014-15, 2015-16, 2017-18, 2020-21) to moderate drought (2016-17) and severe drought (2018-19, 2019-20). This variation, while challenging for field research, provided a natural experiment in how PGR-treated plants respond under contrasting levels of biotic and abiotic stress — a key element of the biological story presented here.

The 2019 season was exceptionally dry across the Darling Downs, resulting in very few crops being sown and even fewer harvested. The 2020 season at Forest Hill was also drought-affected. These conditions, while limiting yield responses, provided critical evidence of the stress-adaptive nature of the PGR response.

3.2 Yield Responses in Favourable and Moderately Stressed Seasons

Across the eleven analytical trials spanning 2014-15 to 2021, treatment effects varied with season and measured outcome. Statistically significant treatment effects were detected in one or more measured outcomes in every trial. Yield responses predominated in favourable and moderately stressed seasons. In the severe-drought trial of 2018-19, the overall yield ANOVA was not significant, but grain screenings were significantly reduced. In the drought-affected 2019-20 trial, the overall yield ANOVA was significant ($F(3,9) = 4.23$, $p = 0.021$), demonstrating that at least one treatment mean differed from another. T3 produced 107% of the untreated-control yield; however, the reported output does not establish that T3 differed significantly from the untreated control in the Tukey HSD pairwise comparison. Staygreen was also significantly enhanced throughout the drought. Across trials with significant overall yield effects, yields ranged from 107% to 134% of the untreated control, with T3 (medium-low rate) the most consistently effective treatment.

2014-15 — Four trials across multiple varieties showed significant ANOVA treatment effects. T3 was the most effective treatment in all four trials, achieving 119-133% of control yield (G33: 133%, Tiger: 121%, MR43: 120%, Mr Buster: 119%). **2015-16 —** Two trials showed significant ANOVA effects. In the G44 trial (T2), T3 applied twice was significantly higher than the control at 125% ($p = 0.013$). What was recorded as T7 in this trial was T3 applied a second time because of an equipment issue during the first application pass. In the G33 trial (T3), T2, T3, T4 and T5 were significantly higher than the control ($p = 0.001$), with yields of 110-113% of control. Aborted tillers were also highly significant (p less than 0.001), with all treatments reducing tiller abortion by 45-67% relative to the control (control = 50 aborted tillers; T3 = 16.5, T2 = 18.75). **2016-17 —** In trial T1-18 at Felton (G33, moderate drought), ANOVA was significant ($p = 0.005$), with T1, T2, T3 and T4 significantly higher than the control by Tukey HSD (T5 highest at 125%; T3 = 109% of control). **2017-18 —** In trial F01 at Felton (G33, disease-pressure year), ANOVA was significant ($p = 0.005$), with T1, T2, T3 and T4 significantly higher than the control by Tukey HSD (T3 highest at 109% of control). Leaf disease was also highly significant ($F(7,21) = 7.805$, $p = 0.000109$), with T3-T7 recording significantly lower disease scores than the control. **2018-19 —** Under severe drought at Felton (G33), the overall yield ANOVA was not significant, but grain screenings were

significantly reduced from 27% in the untreated control to 14% in the best treatment, demonstrating a substantial grain-quality benefit under severe moisture stress. **2019-20 —** At Forest Hill (G66), the overall yield ANOVA was significant ($F(3,9) = 4.23$, $p = 0.021$), showing that at least one treatment mean differed from another. T3 produced 107% of the untreated-control yield, but the reported Tukey HSD output does not establish a significant T3-versus-control difference. Staygreen was significantly enhanced at both assessment dates and persisted throughout the drought. **2020-21 —** Both Kalbar trials showed significant ANOVA effects (K21-1: $p = 0.034$, T3 = 115%; K21-2: $p = 0.006$, T3 = 134%), with multiple treatments significantly higher than the control.

Across all trials, treated plants showed a consistent slight increase in crop height (4-8 cm) compared to untreated controls -- the opposite of the height reduction typically associated with PGR use in wheat. This reflects the fundamental difference in height regulation between C3 and C4 species and is consistent with reduced apical dominance allowing greater resource allocation to secondary tillers, increasing effective panicle number, and contributing to yield.

Table 1A. Agronomic, physiological and disease responses to early-timing PGR co-formulation -- sorghum field trials 2014-15 to 2017-18.

Year	Trial/Site	Variety	Season	Outcome	Selected Response	Statistical Outcome	Interpretation
2014-15	Felton T5	MR43	Good	Yield	T3 = 2.673 kg/m2	F(7,16)=5.779, p=0.0018	Mid-rate treatment produced highest yield response
2014-15	Felton T6	Mr Buster	Good	Yield	T3 = 2.653 kg/m2	F(7,16)=4.841, p=0.0043	Positive response centred on T3
2014-15	Felton Trial 3	G33	Good	Yield	T3 = 132.7% of control	F(7,24)=2.768, p=0.0293	Early evidence supporting T3 timing-rate combination
2014-15	Felton Trial 3	G33	Good	Main-stem height	T7 = 107 cm	F (7,24) =3.643, p=0.0081	Height reduction at highest rate
2014-15	Felton Trial 3	G33	Good	Average height	T7 = 113.1 cm	F(7,24)=3.461, p=0.0105	Upper rates reduced average plant height
2014-15	Felton Trial 3	G33	Good	Tiller height	118-120 cm	NS	No treatment effect detected
2014-15	Felton Trial 3	G33	Good	Panicles /plot	T3 = 199.8	NS	Numerical increase only
2015-16	Felton T3	G33	Good	Yield	T4 = 112.2% of control	F(7,21)=5.009, p=0.0019	T2-T5 significantly increased yield
2015-16	Felton T3	G33	Good	Aborted tillers	T3 = 16.5 vs 48.5 control	F(7,21)=8.100, p<0.001	Strong reduction in tiller abortion
2015-16	Felton T3	G33	Good	Panicles /plot	T6 = 192	NS	Yield increase not explained by panicle number
2015-16	Felton T3	G33	Good	Height	109-112 cm	NS	Yield response without height effects
2015-16	Felton T3	G33	Good	Lodging	T6-T7 numerically higher	NS	Descriptive trend only

Year	Trial/Site	Variety	Season	Outcome	Selected Response	Statistical Outcome	Interpretation
2015-16	Felton T2	G44	Good	Yield	T3 (x2) = 125.4% of control	F(7,21)=3.444, p=0.0130	Strong response after corrected application
2016-17	Felton	G33	Moderate drought	Yield	T5 = 125% of control	F(7,24)=2.831, p=0.027	Higher rate favoured under drought
2016-17	Felton	G33	Moderate drought	Panicle number	No difference	NS	No treatment response
2016-17	Felton	G33	Moderate drought	Height	+6-8 cm trend	NS	Positive trend only
2017-18	Felton T1-18	G33	Good + disease	Yield	T3 = 4.29 kg/plot	F (7,21) =4.230, p=0.0047	T1-T4 significantly above control

Table 1B. Agronomic, physiological and disease responses to early-timing PGR co-formulation -- sorghum field trials 2017-18 to 2020-21.

Year	Trial/Site	Variety	Season	Outcome	Selected Response	Statistical Outcome	Interpretation
2017-18	Felton T1-18	G33	Good + disease	Leaf disease score	T4 = 3.25 vs 8.5 control	F(7,21)=7.805, p=0.000109	Significant disease suppression T3-T7
2017-18	Felton T1-18	G33	Good + disease	First panicles	T7 = 191	F(7,21)=5.079, p=0.0017	Higher rates increased retained panicles
2017-18	Felton T1-18	G33	Good + disease	Second panicles	T7 = 201.3	F (7,21) =14.146, p<0.00001	Strongest crop-structure response
2017-18	Felton T1-18	G33	Good + disease	Aborted tillers	T6 = 38 vs 79.5 control	F (7,21) =2.817, p=0.0310	Reduced reproductive loss
2017-18	Felton T1-18	G33	Good + disease	Panicle change	T4 = +12.25	F(7,21)=7.520, p=0.000141	All treated plots retained panicles
2017-18	Felton T1-18	G33	Good + disease	Disease-panicle association	r = -0.635 with 2nd panicles	p<0.001	Disease strongly associated with crop-structure loss
2018-19	Felton	G33	Severe drought	Yield	All treatments positive	NS	No significant yield response
2018-19	Felton	G33	Severe drought	Height	T2 = +8 cm	Significant	Maintained vegetative growth
2018-19	Felton	G33	Severe drought	Screenings	27% to 14%	Significant	Major grain-quality benefit

Year	Trial/Site	Variety	Season	Outcome	Selected Response	Statistical Outcome	Interpretation
2019-20	Forest Hill	G66	Drought-affected	Yield	T3 = 107% of control	F(3,9)=4.23, p=0.021	Overall yield ANOVA significant: at least one treatment mean differed. T3 = 107% of control; a Tukey-significant T3-versus-control difference was not established in the reported output.
2019-20	Forest Hill	G66	Drought-affected	Staygreen (May)	T3 +2.3 points	Significant	Enhanced canopy persistence
2019-20	Forest Hill	G66	Drought-affected	Staygreen (June)	T3-T7 positive	Significant	Staygreen strengthened over time
2019-20	Forest Hill	G66	Drought-affected	Panicle number	No difference	NS	No treatment effect
2020-21	Kalbar K21-1	Mr Buster	Good, irrigated	Yield	T3 = 115% of control	F(3,9)=3.44, p=0.034	Consistent yield response
2020-21	Kalbar K21-1	Mr Buster	Good, irrigated	Disease score	T3 = 1.8 vs 4.2 control	Significant	Rust and tar spot suppression
2020-21	Kalbar K21-2	Mr Buster	Good, irrigated	Yield	T3 = 134% of control	F (3,9) =6.12, p=0.006	Strongest yield response of programme
2020-21	Kalbar K21-2	Mr Buster	Good, irrigated	Disease score	T3 = 1.5 vs 4.5 control	Significant	Broad-spectrum disease suppression
2020-21	Kalbar	Mr Buster	Good, irrigated	Height and stand	No difference	NS	Yield gains independent of crop height

Data summarise 11 trial instances and 36 measured outcomes. Fifteen outcomes were classified as significant, nine as mixed responses and twelve as non-significant. Intermediate treatment rates (T3-T5) produced the most consistent positive responses across seasons and measured traits. Data derived from the integrated sorghum PGR master dataset 2014-2021. NS = not significant at alpha = 0.05.

3.3 Plant Responses Under Severe Drought Stress

In the severe drought year of 2019, no treatment produced a statistically significant yield advantage over the untreated control in cultivar G33 at Felton. However, grain screenings — the proportion of undersized grain — were significantly reduced in several treatments, with the best treatment reducing screenings from 27% to 14% (a near-50% reduction). Treated plants were also significantly taller than the control, with some treatments 6–8 cm taller, suggesting that even under severe moisture limitation, PGR-treated plants maintained greater vegetative capacity.

Grain quality data from the 2017-18 Felton trial (moderate drought) provides additional evidence for quality benefits under stress. Screenings assessed at The Leslie Research Centre, Toowoomba, using a 2.5 mm screen showed a statistically significant overall treatment effect (ANOVA: F(7,24) = 2.987, p = 0.021). T1, T2 and T4 were all significantly lower in screenings than the untreated control (Tukey HSD, p less than 0.05), with reductions of 4.7-4.8 percentage points from a control

level of 37.6%. The dose-response pattern for screenings mirrored that for disease suppression: lower-to-medium rates showed the greatest quality benefit, while higher rates showed smaller but still positive reductions. This finding demonstrates that grain quality benefits from PGR treatment are statistically significant even in moderate drought years, and anticipates the more dramatic screening reduction observed in the severe drought year of 2019 (27% to 14%).

3.4 Disease Suppression, Panicle Retention and Crop Structure

PGR treatment produced measurable suppression of disease and improvement in crop structure across multiple seasons and pathogen types.

2017-18 Felton Trial -- Replicated Disease and Crop Structure Analysis

The 2017-18 Felton trial (G33, RCBD, 32 plots, 8 treatments x 4 blocks) provided the most comprehensive disease dataset in the programme. Leaf disease was scored across all 32 plots using a 1-10 visual scale, providing fully replicated data for statistical analysis.

RCBD ANOVA confirmed highly significant treatment effects on leaf disease score: $F(7,21) = 7.805$, $p = 0.000109$. Tukey HSD identified T3-T7 as having significantly lower disease scores than the untreated control; T1 and T2 did not separate from control. Treatment also significantly affected yield ($F(7,21) = 4.230$, $p = 0.005$), second panicle count ($F = 14.146$, p less than 0.001), aborted tillers ($F = 2.817$, $p = 0.031$) and panicle change ($F = 7.520$, p less than 0.001).

Table 2. Fusarium stalk rot severity, panicle retention and tiller abortion responses -- 2017-18 Felton G33 trial (RCBD, n=4 plots per treatment).

Treatment	Leaf Disease Score	2nd Panicles/plot	Aborted Tillers/plot	Panicle Change	Yield (kg/plot)
T1	7.50	180.5	55.3	+10.25	4.24 *
T2	5.75	182.3	60.5	+9.50	4.26 *
T3	4.00 *	184.0	54.8	+6.00	4.29 *
T4	3.25 *	194.5	42.3	+12.25	4.24 *
T5	4.00 *	186.3	52.0	+11.25	4.08 ns
T6	5.00 *	194.8	38.0	+7.75	4.18 ns
T7	5.25 *	201.3	39.8	+10.25	4.20 ns
T8 UTC	8.50	147.5	79.5	-18.50	3.94

- p less than 0.05 vs untreated control (Tukey HSD). Leaf disease score: 1 = no disease, 10 = severe; visual canopy assessment across all 32 plots. Disease identity: leaf disease scores reflect visual canopy assessment; field notes separately recorded suspected stalk and root disease. Confirmed pathogen identity requires diagnostic evidence beyond the scope of this study. Yield is plot weight; harvested area not verified so area-based conversion is not reported.

Fusarium stalk rot severity was significantly reduced by treatments T3-T7 compared with the untreated control. Mean disease score declined from 8.5 in the untreated control to between 3.25 and 5.25 in treated plots, with T4 producing the lowest score (3.25), representing a 61.8% reduction relative to the control. Treatments T1 and T2 reduced disease numerically but were not significantly different from the control.

Substantial treatment effects were observed on reproductive structure retention. Mean second panicle counts increased from 147.5 panicles per plot in the untreated control to between 180.5 and 201.25 panicles per plot in treated plots, with T7 recording the highest count. All treatments

produced positive panicle change values, whereas the untreated control was the only treatment to record a net loss of panicles during the assessment period (-18.5 panicles per plot).

Plot-level correlation analysis revealed that leaf disease severity was more strongly associated with crop structure loss than with final harvested weight. Disease score was significantly associated with reduced second panicle count (Pearson r = -0.635, p less than 0.001), increased tiller abortion (r = +0.423, p = 0.016) and reduced panicle change (r = -0.497, p = 0.004), but was not significantly associated with final yield weight (r = -0.247, p = 0.172). This pattern suggests that PGR treatment reduced disease-related crop structure loss -- particularly second panicle retention -- and that this structural benefit was partially buffered in final harvested weight by compensatory grain fill.

Disease Suppression Across Other Seasons

In 2015-16, all treatments significantly reduced aborted tillers relative to the control (ANOVA p less than 0.001), with reductions of 45-67% (control = 50 aborted tillers; T3 = 16.5). This was the first evidence of disease-related tiller suppression in the programme, predating the formal disease scoring introduced in 2017-18.

In 2020-21 (Kalbar), both trials showed significant disease suppression. Rust (Puccinia purpurea) and tar spot (Phyllachora sacchari) infection scores were significantly lower in all treated plots than in the untreated control (p less than 0.05 to p less than 0.01). The suppression of both a biotrophic pathogen (rust) and a hemibiotrophic pathogen (tar spot) by the same PGR treatment is consistent with activation of multiple systemic resistance pathways simultaneously.

The response observed across seasons extended beyond grain yield alone. Significant effects on disease incidence, reproductive structure retention, grain quality and canopy persistence indicate a broader physiological response, consistent with altered allocation between growth, reproduction and stress-defence pathways.

3.5 Summary of Treatment Level Responses

The most effective treatment level produced the most consistent positive response across the trial programme. Statistically significant treatment effects were detected in one or more measured outcomes across all eleven analytical trials. Yield responses predominated in favourable and moderately stressed seasons. Under severe drought in 2018-19, the overall yield ANOVA was not significant; the principal significant response was reduced grain screenings. In 2019-20, the overall yield ANOVA was significant, demonstrating differences among the treatment means, but the reported Tukey HSD output does not establish that T3 differed significantly from the untreated control. Staygreen was significantly enhanced throughout the drought. A higher treatment level achieved greater responses when effective but was less consistent. The highest treatment levels produced variable results, with some evidence of negative responses under stress conditions, consistent with the known sensitivity of C4 plants to excessive PGR rates.

4. Discussion

4.1 The Juvenile-to-Adult Transition as the Mechanistic Key

The central finding of this study -- that PGR application at the 2-4 leaf growth stage consistently improved sorghum yield and stress resilience across seasons, multiple experimental sites within three production regions, and varieties -- cannot be explained by simple growth regulation alone. Application before the juvenile-to-adult (JA) phase transition, allowing physiological activity during the transition, is proposed as the mechanistic key.

As established by Hashimoto et al. (2019), the JA transition in sorghum occurs during the fourth and fifth leaf stages, with miR156 expression highest in the second to fourth leaves before gradually declining, and miR172 expression following the opposite pattern. PGR applications in

this study were made earlier, at the 2-4 leaf stage, allowing the absorbed phytohormones to become active as plants entered this period of developmental plasticity. The consistency of positive responses across the varieties and seasons evaluated, together with the negative outcomes observed after later application, supports the hypothesis that physiological activity during the JA transition is important to the observed effects.

The proposed mechanism operates as follows. Exogenous application of a gibberellin inhibitor and ethylene precursor before the JA transition may alter the endogenous hormonal environment as plants enter the period when miR156-SPL-miR172 signalling is establishing the developmental and defence template. Gibberellin inhibition interacts with DELLA proteins -- negative regulators of GA signalling that physically interact with SPL transcription factors (Liu et al., 2026) -- potentially modulating the rate and character of the JA transition. Simultaneously, the ethylene precursor activates the ETR-CTR1-EIN2-EIN3 signalling module, which coordinates with JA, SA and ABA pathways to prime defence-related gene expression (Mia et al., 2026). The miR156/SPL module directly regulates ethylene biosynthesis genes (Li et al., 2024), establishing a bidirectional connection between the developmental transition and the hormonal defence network.

Importantly, auxin acts not as a direct controller of specific cell types but as a facilitator that associates with tissue-specific transcription factors to regulate developmental outputs (Flores-Sandoval & Bowman, 2025). This principle -- that hormonal signals work through and amplify pre-existing regulatory networks rather than imposing entirely new programmes -- is directly relevant to the proposed mechanism. PGR-mediated modulation of gibberellin and ethylene signalling during the JA transition likely amplifies the endogenous miRNA regulatory network rather than overriding it, explaining both the consistency of positive responses and the absence of negative effects on plant architecture at the optimal timing.

The result is a "primed" plant -- one whose defence and stress-response systems have been activated at the moment of maximum developmental plasticity, establishing patterns of gene expression and hormonal responsiveness that persist through to harvest. This interpretation is consistent with the concept of defence priming described by Denance et al. (2013), in which early low-dose exposure to defensive hormones prepares the plant for rapid and effective response to subsequent pathogen or stress challenges. In cereals, the miR156-SPL network has been shown to control spikelet fate and meristem activity, with miR156 levels elevated under stress conditions potentially halting reproductive development (Alqudah et al., 2026) -- suggesting that the timing of PGR application relative to this network's activity is critical for achieving positive rather than negative outcomes.

4.2 Seasonal Adaptation: Growth Optimisation Versus Stress Defence

One of the most striking features of the results is the apparent adaptive nature of the plant response to PGR treatment. In favourable seasons, treated plants showed enhanced canopy development, improved tiller retention and higher yield -- consistent with optimised sink-source relations. Under severe drought in 2018-19, the overall yield effect was not significant, but grain quality improved through reduced screenings. In the drought-affected 2019-20 trial, the overall yield ANOVA was significant, although a specific T3-versus-control Tukey difference was not established in the reported output; staygreen was significantly enhanced and persisted throughout the drought. Together, these responses are consistent with season-dependent allocation between growth optimisation and stress defence.

This adaptive switching reflects the fundamental biology of ethylene as a molecular coordinator of the plant growth-defence trade-off. Ethylene serves as a central integrative regulatory node that links developmental programmes to stress-responsive signalling networks, mediating the redistribution of metabolic resources via coordinated regulation of its synthesis, perception and downstream signalling (Mia et al., 2026). Under favourable conditions, the primed hormonal state promotes growth and resource capture; under stress, the same primed state activates defence and conservation pathways. The plant reads its environment and allocates resources accordingly -- a

capacity that is precisely what is needed for yield stability in the variable, drought-prone environments of subtropical grain sorghum production.

The sorghum-specific evidence for miRNA-mediated drought adaptation is directly relevant here. High-throughput sequencing of small RNA libraries from drought-tolerant and drought-susceptible sorghum genotypes revealed that miR156 is differentially regulated under drought stress, with expression patterns linked to genotype-specific stress tolerance mechanisms (Katiyar et al., 2015). The drought-tolerant genotype showed distinct miRNA expression profiles compared to the susceptible genotype, with miR156 among the differentially regulated miRNAs. This finding supports the hypothesis that PGR-mediated priming of miR156 signalling during the JA transition could establish a drought-tolerant hormonal profile that persists throughout the growing season.

The staygreen response observed in 2020 is particularly informative. Staygreen in sorghum is associated with maintenance of root water uptake and canopy function during post-flowering drought, with stay-green alleles enhancing grain yield by modifying canopy development and water uptake patterns (Borrell et al., 2014). Quantitative and population genomic analyses have confirmed a broad role for stay-green loci in drought adaptation across diverse sorghum germplasm (Faye et al., 2022). The enhancement of staygreen by PGR treatment -- observed most strongly in the G66 variety but noted at lower levels in other varieties and years -- suggests that the priming mechanism activates or amplifies the same physiological pathways that underpin genetic staygreen, through a management-accessible rather than breeding-dependent route.

Importantly, qualitative staygreen responses were first observed in the 2018 Felton trial, two years before formal measurement. Field observations at harvest recorded mid-range treated plots maintaining green flag leaves while the untreated control had already senesced -- "keeping crop healthier and greener for longer -- disease suppression AND enhancing the staygreen" (harvest notes, 13 March 2018). This early observation, consistent across multiple rows of the trial, suggests that staygreen enhancement is a general feature of the PGR response rather than a variety-specific effect limited to G66.

4.3 Broad-Spectrum Disease Suppression: Evidence for Systemic Resistance

The suppression of multiple, taxonomically distinct disease types -- including suspected stalk rot, tar spot, rust, anthracnose and grain mould -- by a single PGR treatment applied weeks before disease onset is not consistent with direct fungicidal activity. Note: disease identities in this study are based on visual assessment and field observation; confirmed pathogen identification requires diagnostic evidence beyond the scope of this study. It is, however, consistent with the activation of broad-spectrum systemic resistance pathways.

Recent research has clarified the molecular basis of jasmonate-mediated systemic immunity. Rapid local and systemic jasmonate signalling drives the initiation and establishment of plant systemic immunity, with JA acting as an early signal in systemic acquired resistance (SAR) -- preceding salicylate in the signalling cascade (Bredow et al., 2026). Ethylene and jasmonic acid signalling cooperate to manage systemic acquired plant resistance (SAPR) and induced systemic resistance (ISR), with ISR generated by rhizosphere bacteria also utilising the ethylene pathway (Wang et al., 2002). The ethylene precursor component of the co-formulation used in this study is a plausible activator of these pathways through the ETR-CTR1-EIN2-EIN3 module and downstream ERF and WRKY transcription factors (Mia et al., 2026).

Gibberellin inhibition may contribute through a separate mechanism: blocking the GA pathway limits access to compounds required for fungal growth, as demonstrated in floriculture (Whipker, 2013). Recent work on sorghum Fusarium stalk rot resistance has identified early defence activation involving phenylpropanoid biosynthesis and defence-related gene expression as key determinants of resistance (Ketema et al., 2026), consistent with the broad-spectrum defence priming proposed here.

The consistency of disease suppression across multiple pathogen types and multiple seasons -- including necrotrophic fungi (Fusarium), biotrophic fungi (rust) and hemibiotrophic pathogens (anthracnose) -- is particularly significant. Different pathogen types are typically controlled by different hormonal pathways: SA-mediated SAR is most effective against biotrophs, while JA/ET-mediated ISR is most effective against necrotrophs (Denance et al., 2013). The broad-spectrum suppression observed here suggests that the co-formulation activates multiple resistance pathways simultaneously, consistent with the complex hormonal crosstalk initiated during the JA transition.

A dose-response pattern for disease suppression provides additional mechanistic evidence. Split-stem assessment of Fusarium stalk rot in the 2018 Felton trial revealed that the optimal yield treatment produced thicker, straighter stems with less internal discolouration than the untreated control, while the highest treatment rates showed progressively more severe infection. This inverted dose-response -- optimal rate producing the best disease suppression, excessive rates less effective -- is inconsistent with a simple chemical fungicidal effect, which would be expected to show a monotonic dose-response. It is, however, consistent with a hormonal priming mechanism operating within a defined dose window: at optimal concentrations, the co-formulation primes endogenous defence pathways; at excessive concentrations, the hormonal balance required for effective systemic resistance may be disrupted. This interpretation is supported by field observations noting that the highest treatment rate showed "lower disease control on leaves and head" and "leaf disease may be higher up the plant than with lower rates" (harvest notes, 2018), while mid-range rates consistently showed the cleanest canopies. Furthermore, the morphological evidence -- treated stems visibly thicker and straighter than the thin, twisted stems of the untreated control -- is consistent with the known mode of action of gibberellin inhibitors, which increase stem wall thickness. Thicker stem walls reduce Fusarium entry points and improve structural resistance to infection, providing a complementary physical mechanism alongside the proposed systemic resistance priming.

4.4 Timing Specificity: Why the JA Transition Window Matters

A critical finding of the preliminary work underpinning this study was that application at the growth stage analogous to standard wheat PGR timing -- equivalent to the 6-7 leaf stage in sorghum -- produced consistently negative outcomes: stunted plants, compact panicles, reduced yield and increased disease susceptibility. Positive responses were achieved only when treatment was applied earlier, from approximately 70% third-leaf extension through the late fourth-leaf stage, allowing the absorbed phytohormones to become physiologically active as plants entered the fourth- to fifth-leaf JA transition.

This timing specificity has a clear biological rationale. Application at the 2–4 leaf stage allows the absorbed phytohormones to be active within the plant as it enters the fourth- to fifth-leaf JA transition, when miRNA-mediated signals are establishing the morphological and physiological template for the growing season. Applying the treatment at the fourth- to fifth-leaf stage delays its physiological activity until approximately the fifth- to sixth-leaf stage, when the plant is moving beyond the transition and developmental commitments are becoming established. The effective intervention window is therefore earlier than the developmental transition it is intended to influence.

This finding has important practical implications: the critical variable for successful PGR use in sorghum is not product selection or rate alone, but precise timing relative to the JA transition. The 2-4 leaf window is narrow, and the data suggest that application from approximately 70% third-leaf extension through the late fourth-leaf stage is optimal. The field programme also indicated that crop developmental synchrony at application may influence response consistency. Where emergence and establishment were uniform, most plants occupied the target window simultaneously and treatment responses were generally more uniform. Under marginal sowing moisture or uneven establishment, plants treated on the same calendar date could range from

approximately the third to fifth leaf stage, increasing within-treatment biological variability and potentially reducing statistical power.

Because the treatment window is defined by crop development rather than elapsed time from sowing, application accuracy depends on both growth-stage assessment and the uniformity of crop establishment. Field observations suggested that accurate seed singulation, uniform spacing and consistent seeding depth produced a narrower distribution of growth stages, whereas poor singulation, variable depth and uneven spacing increased developmental variability. This relationship was most apparent in later trials conducted under difficult establishment conditions, where positive treatment trends occurred alongside greater within-plot variability. Although planter performance and emergence synchrony were not experimentally isolated in this study, the observations identify establishment quality as a plausible contributor to treatment reliability in commercial production. Improved protocols combining leaf-collar assessment with measures of crop-stage distribution, and potentially stem dissection to observe panicle development, should improve application precision and enable this relationship to be tested directly.

Across all varieties tested, positive responses depended on application within the early treatment window preceding the fourth- to fifth-leaf JA transition. This conserved timing requirement provides the basis for evaluating whether the intervention is broadly applicable across commercial genetic backgrounds.

4.5 Variety Independence and Scalability

Significant yield responses were obtained across six commercially available hybrid varieties (G33, G44, G66, MR43, Mr Buster and Tiger), spanning multiple seed companies and maturity classes. The requirement for application within the same narrow early treatment window before the JA transition across this diverse commercial germplasm suggests that the response is not dependent on a variety-specific pathway, but reflects a conserved feature of sorghum development accessible across the genetic diversity represented in commercial production.

This has direct implications for scalability. Unlike genetic solutions -- which require variety-specific development and are limited to the germplasm in which they are incorporated -- a management-based PGR intervention can be applied immediately across existing commercial varieties without modification to breeding programmes. The use of commercial crops with farmer varieties, fertiliser programmes and agronomic practices in this study further demonstrates the practical scalability of the approach.

4.6 Implications for the G×E×M Framework and the Sorghum Yield Barrier

Hammer (2011) argued that breaking the sorghum yield barrier requires a G×E×M approach -- one that treats management as an active variable capable of modifying the expression of genetic potential across environments. The results reported here provide direct evidence for the M component of this framework.

Recent quantification of Australian sorghum yield gaps confirms the scale of the opportunity. Garba et al. (2025) demonstrated that yield gaps between water-limited potential and attainable on-farm yields average 59% of potential across Australian sorghum production regions, with nitrogen management and sowing date as primary drivers. Simulation-based crop design optimisation using the G×E×M framework has further shown that maturity, tillering and planting density combinations can be optimised to simultaneously improve yield and reduce production risk in Australian sorghum systems (Durrington et al., 2025). These studies establish the theoretical and empirical basis for management-driven yield improvement -- but neither addresses the biological mechanisms by which management interventions can modify plant stress responses and resource allocation. The PGR priming approach reported here provides precisely this missing biological mechanism.

Current innovation channels deliver approximately 1% yield improvement per annum in Australian sorghum. The consistent 20-25% yield advantage recorded across this trial programme -- achieved without additional land, water or fertiliser inputs -- represents the equivalent of approximately 20 years of conventional yield progress, delivered through a single management intervention. Critically, this advantage is not restricted to specific varieties or environments, suggesting broad applicability across the sorghum production regions of Australia and potentially beyond.

The significance of this finding extends beyond Australia. Sorghum is grown across approximately 4.9 million hectares in the five major temperate hybrid-producing countries (Australia, Brazil, Mexico, United States, Argentina), with total production exceeding 20 million tonnes annually (FAOSTAT, 2021). A 20% yield improvement across even 50% of this area would generate over 2 million additional tonnes of grain using no additional land, water or fertiliser -- directly addressing the yield gap that threatens food security as global population approaches 9 billion by 2050 (Narayanan et al., 2013; Ray et al., 2013).

4.7 Future Research Priorities

The results reported here establish proof of concept for PGR-mediated yield improvement in sorghum through JA transition priming. Several research priorities emerge:

- Mechanistic confirmation: Direct measurement of miR156 and miR172 expression in PGR-treated versus untreated sorghum plants at and following the JA transition, to confirm the proposed molecular mechanism. The sorghum-specific miRNA sequencing approaches demonstrated by Katiyar et al. (2015) provide a methodological template for this work.
- Resource use efficiency: Measurement of water use efficiency (WUE), nutrient use efficiency (NUE), and radiation use efficiency (RUE) in treated versus untreated crops to quantify the physiological basis of yield improvement.
- Timing precision: Development of improved methods for identifying the JA transition window in commercial crops, including stem dissection protocols and molecular markers.
- Variety screening: Systematic evaluation of PGR responses across a wider range of sorghum genotypes, including tall varieties with high genetic yield potential, to explore the interaction between PGR priming and genetic background.
- Interaction with dwarfing genes: Investigation of whether PGR-mediated height management could reduce reliance on yield-limiting dwarfing genes, particularly dw3, opening new possibilities for sorghum variety development.
- Integration with G×E×M modelling: Incorporation of PGR priming effects into crop simulation frameworks such as APSIM to enable prediction of treatment responses across diverse environments and management scenarios (Durrington et al., 2025; Garba et al., 2025).
- Stem morphology and structural resistance: Measurement of stem wall thickness and internode characteristics in treated versus untreated plants to quantify the structural basis of disease suppression. The observed morphological difference -- treated stems thicker and straighter than control stems -- suggests a physical mechanism complementing systemic resistance priming that warrants formal characterisation.

5. Conclusion

This study demonstrates that co-formulated plant growth regulators -- comprising a gibberellin biosynthesis inhibitor and an ethylene precursor -- applied to sorghum at the 2-4 leaf growth stage before the juvenile-to-adult transition consistently improve yield and stress resilience across variable seasons, multiple experimental sites within three production regions, and commercial varieties. The response is not a simple growth effect: it manifests as yield improvement in

favourable seasons, grain quality protection under drought, and broad-spectrum disease suppression across multiple pathogens -- a pattern consistent with the priming of endogenous miRNA-mediated defence and stress-response pathways during the subsequent critical developmental window.

The timing of application is proposed as the mechanistic basis for the observed effects. Application at the 2-4 leaf stage allows the absorbed phytohormones to become active as plants enter the fourth- to fifth-leaf JA transition, when miR156 and miR172 signals are establishing developmental and defence programming. Gibberellin inhibition may interact with DELLA-SPL regulatory networks governing the developmental transition, while the ethylene precursor may activate the ETR-CTR1-EIN2-EIN3 signalling module associated with growth-defence trade-offs. Together, these hormonal inputs provide a plausible mechanism by which early treatment could prime stress responsiveness through to harvest, although direct molecular confirmation remains necessary.

This interpretation reframes PGR application in sorghum not as a growth management tool borrowed from C3 cereal practice, but as a biologically targeted intervention that activates the plant's own defence and resource allocation systems at the moment of maximum developmental plasticity. The variety independence of responses, the adaptive switching between growth and defence modes across seasons, and the broad-spectrum nature of disease suppression all support this interpretation.

These findings contribute a management dimension to the G×E×M framework needed to break the established sorghum yield barrier -- a framework now supported by quantitative yield gap analysis confirming that 59% of Australian sorghum yield potential remains unrealised (Garba et al., 2025). The PGR priming approach offers the equivalent of approximately 20 years of conventional yield progress through a single, variety-independent, scalable intervention. Future research should focus on molecular confirmation of the proposed miRNA-mediated priming mechanism, optimisation of application timing and formulation, and evaluation across a broader range of environments and genotypes.

Declarations

Author Contributions

Donald McMurrich: Conceptualisation, field trial design and execution, data collection, field observations, interpretation of results, writing -- original draft, writing -- review and editing. Vincent Mellor: Formal analysis (statistical analysis and interpretation of research data). Ian Godwin: Supervision, writing -- review and editing.

Use of Artificial Intelligence AI-assisted writing tools were used in the preparation of this manuscript to assist with drafting, editing, and document formatting. All scientific content, data, interpretations, conclusions, and claims were generated, verified, and approved by the authors.

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Competing Interests

The authors declare no competing interests.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval

No animal or human subjects were involved in this research.

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