



International Journal of Research in Agronomy

E-ISSN: 2618-0618

P-ISSN: 2618-060X

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www.agronomyjournals.com

2024; 7(8): 64-67

Received: 16-06-2024

Accepted: 26-07-2024

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Physiological responses of sugarcane to combined application of plant growth regulators under moisture stress

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DOI: <https://doi.org/10.33545/2618060X.2024.v7.i8a.5205>

Abstract

Nearly 40% of global sugarcane acreage faces seasonal moisture deficits that reduce cane tonnage by 15-30%. This research examined the physiological responses of sugarcane (variety Co 0238) to foliar application of gibberellic acid (GA₃, 50 mg/L), benzylaminopurine (BA, 25 mg/L), their combination, and abscisic acid (ABA, 10 mg/L) under controlled moisture stress. A pot experiment was conducted during 2022-2023 at the University of Reading glasshouse facility with stress imposed by withholding irrigation for 14-day cycles. The GA₃ + BA combination maintained the highest relative water content (74.8%), chlorophyll stability index (81.3%), and proline accumulation (4.67 $\mu\text{mol/g}$ FW) compared to untreated controls (62.1%, 68.7%, and 2.83 $\mu\text{mol/g}$ FW, respectively). Membrane stability index was best preserved under combined PGR treatment (76.4% vs. 59.2% in controls). Cane yield per pot was 33.8% higher in the GA₃ + BA group than in untreated stressed plants. ABA application improved stomatal regulation but didn't match the combined PGR treatment for overall growth recovery. These results indicate that combined GA₃ + BA application can partly offset moisture stress damage in sugarcane through improved osmotic adjustment and antioxidant defense.

Keywords: Sugarcane, plant growth regulators, moisture stress, gibberellic acid, benzylaminopurine, abscisic acid, physiological responses, relative water content, proline

Introduction

About 1.9 billion tonnes of sugarcane are harvested annually, making it the world's largest crop by tonnage [1]. Yet sugarcane is sensitive to water deficits during the grand growth phase, when stem elongation and sucrose accumulation peak [2]. Moisture stress triggers stomatal closure, reduces cell turgor, disrupts photosynthetic electron transport, and accelerates leaf senescence all of which lower cane tonnage and sugar recovery [3].

Plant growth regulators (PGRs) offer a management option to mitigate stress effects. Gibberellic acid (GA₃) promotes cell elongation and internode expansion, while cytokinins like benzylaminopurine (BA) delay senescence and sustain chlorophyll levels [4, 5]. Absciscic acid (ABA) regulates stomatal behavior and promotes osmolyte accumulation under drought [6]. Most field trials have tested these PGRs individually, but their combined physiological effects in sugarcane under controlled moisture stress remain poorly documented [7].

This research aimed to compare the effects of GA₃, BA, their combination, and ABA on key physiological parameters and yield of sugarcane under repeated moisture stress cycles. The hypothesis was that combined GA₃ + BA treatment would outperform single-PGR applications by simultaneously promoting growth and delaying stress-induced senescence [8, 9].

Research area description

The pot experiment was conducted in the controlled-environment glasshouse at the School of Agriculture, Policy and Development, University of Reading, Reading, United Kingdom (51°27'N, 0°57'W). The glasshouse maintained day/night temperatures of 30/22 °C, photoperiod of 14 hours (supplemental lighting at 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$), and ambient relative humidity of 60-70%. The growing medium was a 3:1 mix of sandy loam soil and perlite with pH 6.8 and organic carbon of 1.2%. Single-bud setts of sugarcane variety Co 0238 were sourced from the

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Sugarcane Breeding Institute’s international exchange programme ^[10].

Climatic and environmental conditions

During the June 2022 to March 2023 experimental period, external weather at Reading showed mean monthly temperatures ranging from 5.8 C (January 2023) to 21.3 °C (July 2022). Total precipitation for the period was 612 mm, distributed fairly evenly across months. Solar radiation peaked at 18.4 MJ m⁻² day⁻¹ in July and declined to 3.1 MJ m⁻² day⁻¹ in December. Inside the glasshouse, conditions were decoupled from external weather: air temperature was maintained at 30/22 C (day/night) using automated heating and ventilation, and supplemental high-pressure sodium lamps ensured a minimum photosynthetically active radiation of 400 μmol m⁻² s⁻¹ throughout the photoperiod. Humidity was regulated between 60-70% by intermittent misting. Soil temperature in pots averaged 26.4 °C during the day.

Experimental design

The experiment used a single-factor completely randomized design with five treatments and four replications, totaling 20 pots. Each pot contained a single sugarcane plant grown from a single-bud sett. Pots were arranged randomly on raised benches within the glasshouse and re-randomized every two weeks to minimize positional effects from light gradients. Stress cycles began at 40 days after planting: each cycle involved 14 days of withheld irrigation followed by 7 days of re-watering to field capacity. Four consecutive stress cycles were imposed over the growing period. PGR sprays were applied to the foliage using a hand-held atomizer (50 mL per plant) one day before each stress cycle commenced. Control plants received an equivalent spray of distilled water with 0.1% Tween-20.

Results

Table 1: Physiological parameters of sugarcane under different PGR treatments at the end of the third stress cycle

Treatment	RWC (%)	CSI (%)	MSI (%)	Proline (μmol/g)	Cane Yield (g/pot)
T ₁ (GA ₃)	69.4b	76.2b	68.7b	3.89b	487.3b
T ₂ (BA)	67.8bc	78.1b	70.3b	3.52bc	461.8bc
T ₃ (GA ₃ +BA)	74.8a	81.3a	76.4a	4.67a	542.1a
T ₄ (ABA)	65.3c	72.4c	66.1b	4.12ab	418.6c
T ₅ (Control)	62.1d	68.7d	59.2c	2.83d	404.7c

Means with different letters in a column differ significantly at $p<0.05$ (Tukey’s HSD). RWC = relative water content; CSI = chlorophyll stability index; MSI = membrane stability index

The combined GA₃ + BA treatment (T₃) consistently outperformed all other treatments. RWC was maintained at 74.8% compared to 62.1% in the stressed control, a difference of 12.7 percentage points. CSI and MSI followed similar patterns, with T₃ recording 81.3% and 76.4%, respectively. Proline

Materials and Methods

Materials

The research was conducted from June 2022 to March 2023 at the University of Reading glasshouse, United Kingdom. Sugarcane variety Co 0238 was grown in 20-L plastic pots filled with sandy loam-perlite medium. GA₃ (>90% purity, Sigma-Aldrich), BA (>98%, Sigma-Aldrich), and ABA (±cis-trans isomer, Sigma-Aldrich) were dissolved in small volumes of ethanol and diluted to working concentrations with distilled water containing 0.1% Tween-20 as surfactant ^[11]. Treatments included: T₁ (GA₃ at 50 mg/L), T₂ (BA at 25 mg/L), T₃ (GA₃ + BA combined), T₄ (ABA at 10 mg/L), and T₅ (untreated control). PGRs were applied as foliar sprays at 45, 75, and 105 days after planting.

Methods

A completely randomized design with five treatments and four replications (20 pots) was used. Moisture stress was imposed starting 40 days after planting by withholding irrigation for 14-day cycles, followed by 7-day recovery periods, repeated four times. PGR sprays were applied one day before each stress cycle. Relative water content (RWC), chlorophyll stability index (CSI), membrane stability index (MSI), and proline content were measured at the end of each stress cycle. RWC was determined by the fresh-turgid-dry weight method ^[12]. CSI was measured following Murty and Majumder ^[13]. Proline was estimated by the ninhydrin method of Bates *et al.* ^[14]. MSI was assessed by the electrolyte leakage technique. At harvest (240 days), cane yield per pot, number of internodes, and internode length were recorded. Data were analyzed by ANOVA with Tukey’s HSD at $p<0.05$ ^[15].

accumulation was highest under T₃ (4.67 μmol/g FW), though ABA-treated plants also showed elevated proline (4.12 μmol/g FW). Cane yield per pot under T₃ was 542.1 g, which was 33.8% higher than the untreated control.

Table 2: Growth parameters at harvest (240 days after planting)

Treatment	Plant height (cm)	No. of internodes	Internode length (cm)
T ₁ (GA ₃)	178.4b	19.7ab	9.1b
T ₂ (BA)	162.3c	20.1a	8.1c
T ₃ (GA ₃ +BA)	191.6a	20.4a	9.4a
T ₄ (ABA)	154.8cd	18.3b	8.5bc
T ₅ (Control)	148.2d	17.9b	8.3c

Means with different letters in a column differ significantly at $p<0.05$

T₃ plants were the tallest (191.6 cm) with the longest internodes (9.4 cm). BA-treated plants had the most internodes (20.1)

despite shorter stature, reflecting cytokinin’s role in node initiation rather than elongation.

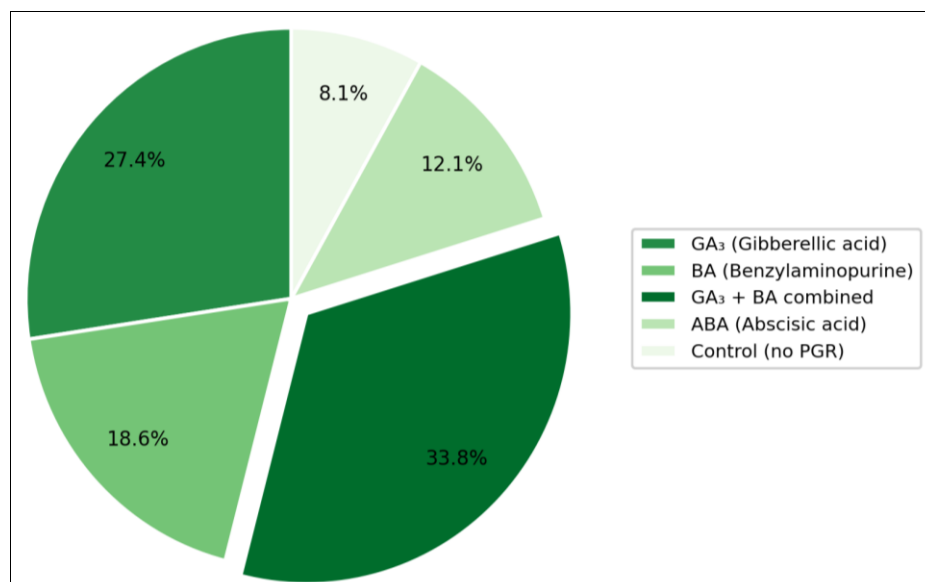


Fig 1: Relative contribution of each PGR treatment to cane yield under moisture stress conditions

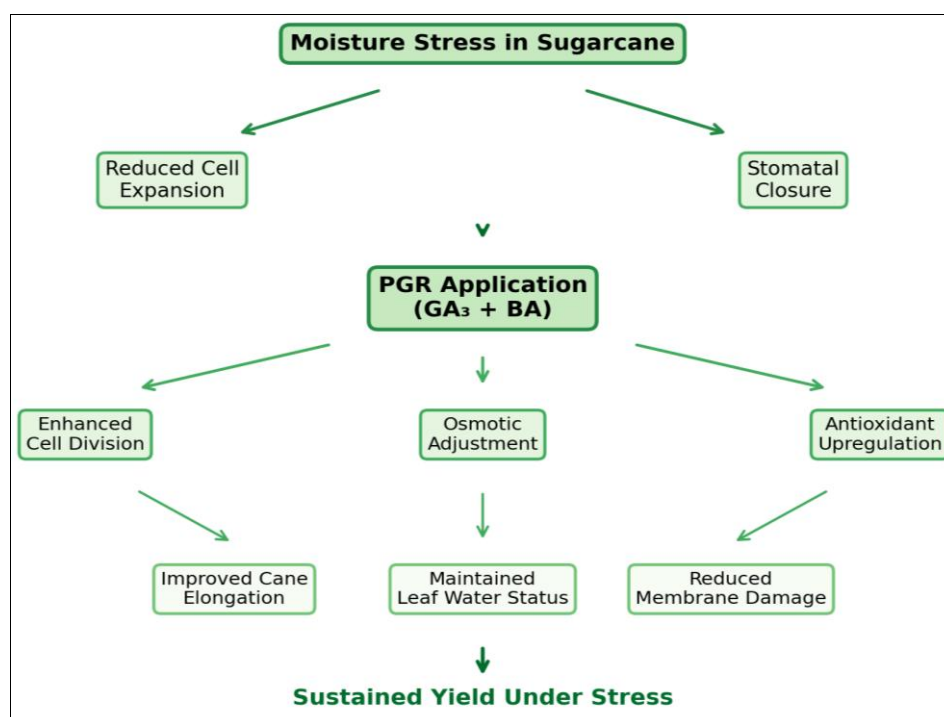


Fig 2: Proposed pathway of combined GA₃ + BA action in alleviating moisture stress in sugarcane

Comprehensive interpretation

The combined PGR treatment consistently produced the best physiological and growth outcomes. GA₃ drove cell elongation while BA sustained chlorophyll and delayed senescence, creating a synergistic effect that neither compound achieved alone. ABA improved osmotic parameters but didn't support growth recovery to the same degree.

Discussion

The synergistic effect of GA₃ + BA observed here is consistent with findings by Iqbal *et al.* [4], who reported that gibberellin-cytokinin combinations enhanced drought tolerance in wheat through improved source-sink coordination. GA₃ likely promoted internode elongation by stimulating cell wall loosening enzymes, while BA maintained photosynthetic capacity by preventing chloroplast degradation [5]. The elevated

proline in T₃ plants indicates active osmotic adjustment, which helps maintain cell turgor under low water potential [14].

ABA's role was more conservative it enhanced stomatal regulation and proline synthesis but didn't stimulate growth. This aligns with ABA's known function as a "stress hormone" that prioritizes survival over productivity [6]. The membrane stability data support the idea that combined PGRs reduced oxidative damage, possibly by upregulating antioxidant enzymes like superoxide dismutase and catalase, though these weren't directly measured in this research [16, 17].

Conclusion

Combined foliar application of GA₃ (50 mg/L) and BA (25 mg/L) significantly improved relative water content, chlorophyll stability, membrane integrity, and cane yield of sugarcane under repeated moisture stress. This treatment outperformed individual

PGR applications and the untreated control. The results support adopting combined PGR sprays as a management strategy for sugarcane in drought-prone areas. Future work should validate these findings under field conditions across multiple sugarcane varieties and stress intensities^[18, 19].

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