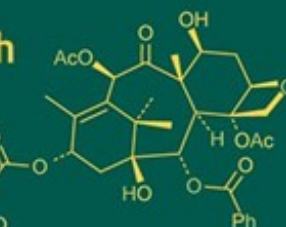


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
Indexing: Embase
IJABR 2026; SP-10(9): 2308-2315
www.biochemjournal.com
Received: 17-07-2026
Accepted: 19-08-2026
Published: 26-09-2026

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Phenology-synchronized nano-biostimulant consortia enhance productivity and multi-nutrient-use efficiency of early-, medium and late-maturing maize (*Zea mays* L.) hybrids under contrasting nitrogen environments

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i9Sac.10168>

ABSTRACT

Nitrogen management in modern maize (*Zea mays* L.) production systems is complicated by hybrid-specific differences in phenological duration and nutrient-demand dynamics, while the integration of biostimulants into precision, phenology-based nutrient schedules remains poorly characterized. A field experiment was conducted during the 2025–26 kharif season at BEST Innovation University, Andhra Pradesh, India, in a Randomized Block Design with three replications, to evaluate the interactive effects of three maize hybrid maturity groups (early, H₁; medium, H₂; late, H₃), two nitrogen environments (low-N, 60 kg N ha⁻¹; recommended-N, 120 kg N ha⁻¹) and a phenology-synchronized ternary nano-biostimulant consortium (seaweed extract + plant growth-promoting rhizobacteria + humic acid encapsulated in a chitosan nanocarrier; B₀ = control, B₁ = nano-consortium applied at the V6, VT and R2 stages of each hybrid) on growth, yield, grain quality, soil nutrient dynamics and multi-nutrient-use efficiency. Grain yield increased significantly with hybrid maturity duration (3973, 4942 and 5936 kg ha⁻¹ for H₁, H₂ and H₃, respectively), nitrogen level (5770 vs. 4130 kg ha⁻¹) and nano-biostimulant application (5356 vs. 4545 kg ha⁻¹; +17.8%), with the highest yield (7724 kg ha⁻¹) recorded for the late-maturing hybrid under recommended nitrogen with the nano-consortium (T₁₂) — a 145.3% gain over the lowest-yielding treatment. Significant hybrid × biostimulant interactions for grain yield and for partial factor productivity of nitrogen, phosphorus-use efficiency and potassium-use efficiency indicated that the absolute magnitude of the biostimulant response scaled with hybrid maturity duration, being largest in the late-maturing hybrid. Nano-biostimulant application did not alter the calendar duration to physiological maturity, and it significantly enhanced plant height, leaf area index, SPAD chlorophyll index, kernel weight, grain protein content, soil available phosphorus and potassium, and microbial biomass carbon. These results demonstrate that stage-scouted, phenology-synchronized nano-biostimulant application is an agronomically robust strategy for improving maize productivity and nutrient-use efficiency, with the largest returns obtained in longer-duration hybrids under adequate nitrogen supply.

Keywords: *Zea mays*, Nano-biostimulant, Chitosan nanoencapsulation, Nitrogen-use efficiency, Hybrid maturity group, Phenology-synchronized application, Seaweed extract, PGPR

1. Introduction

Maize (*Zea mays* L.) is among the most productive and versatile cereal crops in the world, with global production exceeding 1,148 million tonnes harvested from approximately 205 million hectares (FAO, 2023) [1]. As a C₄ crop with a highly efficient carbon-assimilation pathway, maize possesses substantial genetic yield potential; however, realization of this potential depends strongly on the synchronization of nutrient availability, particularly nitrogen (N), with crop developmental demand. In India, maize occupies nearly 10.0 million hectares with an average productivity of only about 3.2 Mg ha⁻¹, well below the 8–12 Mg ha⁻¹ attainable under optimum management, reflecting a substantial and persistent yield gap (Cassman *et al.*, 2020) [2].

Nitrogen demand in maize is highly dynamic, rising sharply from the six-leaf (V6) stage through tasseling (VT) as canopy establishment accelerates, and continuing through grain filling via a combination of continued root uptake and remobilization of pre-silking N reserves. Commercial hybrids differ substantially in phenological duration - broadly classified as early- (80–90 days after planting, DAP), medium- (95–105 DAP) and late-maturing (115–130 DAP) - and, consequently, in the timing, intensity and duration of their nutrient-demand windows (Ciampitti *et al.*, 2012; Bender *et al.*, 2013) [4, 3]. A single calendar-based N schedule may therefore be poorly synchronized with the physiological demand of hybrids possessing different maturity durations, motivating a phenology-based, stage-scouted approach to nutrient and biostimulant management rather than one driven purely by calendar days.

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Biostimulants - substances or microorganisms capable of stimulating natural physiological processes independent of their nutrient content (du Jardin, 2015) [5] - have emerged as a rapidly expanding complement to conventional fertilization. Seaweed extracts (e.g., *Ascophyllum nodosum*) contribute auxin- and cytokinin-like bioactivity that promotes root growth and delays senescence (Battacharya et al., 2015; Shukla et al., 2019) [6, 7]; plant growth-promoting rhizobacteria (PGPR) such as *Azospirillum brasilense* and *Bacillus subtilis* enhance rhizosphere nutrient mobilization and biological nitrogen fixation (Bashan et al., 2014) [8]; and humic substances improve root architecture and nutrient transport (Nardi et al., 2021) [9]. Encapsulation of such bioactive consortia within chitosan-based nanocarriers has additionally been reported to improve compound stability, controlled release and physiological uptake efficiency (Oliveira et al., 2016) [10]. Despite growing interest in both precision nitrogen management and biostimulant technology, limited evidence exists on whether the effectiveness of biostimulant application, when synchronized with hybrid-specific phenological stages rather than fixed calendar dates, differs systematically across maize maturity groups and nitrogen environments. This study therefore examined the combined and interactive effects of hybrid maturity group (early, medium, late), nitrogen environment (low vs. recommended) and a phenology-synchronized ternary nano-biostimulant consortium (seaweed extract + PGPR + humic acid in a chitosan nanocarrier) on growth, phenology, yield, grain quality, soil nutrient dynamics and multi-nutrient-use efficiency of maize, with the specific objectives of (i) quantifying the main and interactive effects of these three factors on productivity and nutrient-use efficiency, and (ii) determining whether the magnitude of the biostimulant response scales with hybrid phenological duration.

2. Materials and Methods

2.1 Experimental site and soil characterization

The field experiment was conducted during the kharif season of 2025–26 at the Research Farm of BEST Innovation University, located in the semi-arid (Köppen BSh) agro-climatic zone of Andhra Pradesh, India. Mean annual rainfall in the region ranges from approximately 700–900 mm, concentrated in the June–September south-west monsoon, and mean kharif-season temperatures range from approximately 24–36 °C. Composite soil samples ($n = 12$, 0–30 cm depth) were analysed for baseline physico-chemical properties prior to treatment imposition (Table 1). The soil was a non-saline (EC 0.32

± 0.04 dS m^{-1}), moderately alkaline (pH 7.8 ± 0.1) sandy clay loam Alfisol with low organic carbon (4.6 ± 0.6 g kg^{-1}) and available nitrogen (178 ± 16 kg ha^{-1}), moderate available phosphorus (20.8 ± 2.8 kg ha^{-1}) and potassium (240 ± 22 kg ha^{-1}), and marginally low available zinc (0.38 ± 0.05 mg kg^{-1}).

2.2 Plant material and treatments

Three commercial maize hybrids representing contrasting maturity groups were used: early-maturing (H_1 , 80–90 DAP), medium-maturing (H_2 , 95–105 DAP) and late-maturing (H_3 , 115–130 DAP). All hybrids were sown on the same date at a spacing of 60 cm $\times$ 25 cm (target population $\approx 60,000$ plants ha^{-1}), with uniform basal application of phosphorus (60 kg P_2O_5 ha^{-1}) and potassium (40 kg K_2O ha^{-1}) to all plots. Nitrogen was applied at two levels - low-N (N_1 , 60 kg N ha^{-1}) and recommended-N (N_2 , 120 kg N ha^{-1}) - constituting the second experimental factor. Biostimulant treatment (Factor C) comprised a water-sprayed control (B_0) and the nano-biostimulant consortium (B_1), applied as a foliar spray at 500 L ha^{-1} using a calibrated knapsack sprayer at three phenological stages of each respective hybrid: V6 (six fully expanded leaves), VT (tasseling/anthesis) and R2 (blister stage). Because the three hybrids differed in developmental rate, field scouting was used to identify the true physiological stage of each hybrid rather than applying treatments on fixed calendar dates, ensuring that biostimulant delivery was synchronized with comparable developmental states across maturity groups.

2.3 Nano-biostimulant consortium: preparation and characterization

The ternary nano-biostimulant consortium comprised *Ascophyllum nodosum* seaweed extract (5% v/v), a PGPR consortium of *Azospirillum brasilense* and *Bacillus subtilis* ($\approx 10^8$ CFU mL^{-1}), and potassium humate (2% w/v), encapsulated within a medium-molecular-weight chitosan nanocarrier (190–310 kDa; degree of deacetylation $\geq 75\%$). Chitosan nanoparticles were synthesized by the ionic-gelation technique using sodium tripolyphosphate (TPP) as the cross-linking agent, with the bioactive components incorporated during nanoparticle formation under controlled mixing to preserve PGPR viability. The formulation was characterized for particle size and zeta potential (Zetasizer Nano ZS, Malvern Panalytical; target zeta potential +25 to +35 mV for colloidal stability), encapsulation efficiency (UV–Vis spectrophotometry), PGPR viability (plate-count on Luria–Bertani agar) and retention of seaweed-extract bioactive constituents (HPLC) prior to field application.

Table 1. Baseline physico-chemical properties of the experimental soil (mean $\pm$ SE, $n = 12$ composite samples, 0–30 cm depth).

Soil property	Value	Method
pH (1:2.5 soil–water)	7.8 ± 0.1	Glass electrode (Jackson, 1973)
Electrical conductivity (dS m^{-1})	0.32 ± 0.04	Conductivity bridge
Organic carbon (g kg^{-1})	4.6 ± 0.6	Walkley–Black
Available N (kg ha^{-1})	178 ± 16	Alkaline $KMnO_4$
Available P (kg ha^{-1})	20.8 ± 2.8	Olsen
Available K (kg ha^{-1})	240 ± 22	Flame photometry
Available Zn (mg kg^{-1})	0.38 ± 0.05	DTPA (Lindsay & Norvell)
Bulk density (Mg m^{-3})	1.45 ± 0.04	Core sampling
Texture	Sandy clay loam	Hydrometer (USDA)
CEC (cmol(p^+) kg^{-1})	14.2 ± 1.3	Ammonium acetate saturation

2.4 Experimental design and treatment structure

The experiment followed a Randomized Block Design (RBD) with a $3 \times 2 \times 2$ factorial treatment structure - Hybrid Maturity Group (H_1 , H_2 , H_3) $\times$ Nitrogen Environment (N_1 , N_2) $\times$ Biostimulant Treatment (B_0 , B_1) - replicated three times (12 treatment combinations $\times$ 3 replications = 36 plots). Each plot measured 4.0 m $\times$ 5.0 m (20 m^2 gross), with a net harvestable area of 3.0 m $\times$ 4.0 m (12 m^2) after excluding a

0.75 m border on all sides. Treatment codes and combinations are summarized in Table 2.

2.5 Observations and analytical procedures

Growth attributes (plant height, stem girth, leaf area index via LI-3100C, dry-matter accumulation, SPAD chlorophyll index, NDVI, and phenological staging) were recorded at V6, VT, R2/R4 and physiological maturity. Yield attributes (cobs plant $^{-1}$, cob length and girth, kernel rows cob $^{-1}$, kernels row $^{-1}$, 100-kernel weight), grain and

stover yield, and harvest index were recorded at harvest. Grain protein was determined by Kjeldahl N $\times$ 6.25. Soil mineral N ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$; 2M KCl extraction), available P (Olsen) and K (flame photometry), and microbial biomass carbon (chloroform fumigation–extraction) were determined at harvest. Multi-nutrient-use efficiency

was computed as partial factor productivity of nitrogen ($\text{PFPN} = \frac{\text{grain yield}}{\text{N applied}}$), nitrogen harvest index ($\text{NHI} = \frac{\text{grain N}}{\text{grain N} + \text{stover N}} \times 100$), phosphorus-use efficiency ($\text{PUE} = \frac{\text{grain yield}}{\text{P applied}}$) and potassium-use efficiency ($\text{KUE} = \frac{\text{grain yield}}{\text{K applied}}$).

Table 2. Treatment structure of the $3 \times 2 \times 2$ factorial experiment (RBD, three replications).

Code	Hybrid maturity	N environment	Biostimulant
$T_1 - \text{H1N1B0}$	Early (H_1)	Low N (60 kg ha^{-1})	Control
$T_2 - \text{H1N1B1}$	Early (H_1)	Low N (60 kg ha^{-1})	Nano-consortium
$T_3 - \text{H1N2B0}$	Early (H_1)	Recommended N (120 kg ha^{-1})	Control
$T_4 - \text{H1N2B1}$	Early (H_1)	Recommended N (120 kg ha^{-1})	Nano-consortium
$T_5 - \text{H2N1B0}$	Medium (H_2)	Low N (60 kg ha^{-1})	Control
$T_6 - \text{H2N1B1}$	Medium (H_2)	Low N (60 kg ha^{-1})	Nano-consortium
$T_7 - \text{H2N2B0}$	Medium (H_2)	Recommended N (120 kg ha^{-1})	Control
$T_8 - \text{H2N2B1}$	Medium (H_2)	Recommended N (120 kg ha^{-1})	Nano-consortium
$T_9 - \text{H3N1B0}$	Late (H_3)	Low N (60 kg ha^{-1})	Control
$T_{10} - \text{H3N1B1}$	Late (H_3)	Low N (60 kg ha^{-1})	Nano-consortium
$T_{11} - \text{H3N2B0}$	Late (H_3)	Recommended N (120 kg ha^{-1})	Control
$T_{12} - \text{H3N2B1}$	Late (H_3)	Recommended N (120 kg ha^{-1})	Nano-consortium

2.6 Statistical analysis

Data were analysed by three-factor factorial ANOVA appropriate to the RBD (Hybrid $\times$ N-environment $\times$ Biostimulant) using Python (statsmodels v0.14), cross-verified in R v4.3.2 (R Core Team, 2024) [11]. Treatment means were compared using critical difference (CD) at $P = 0.05$, computed as $\text{CD} = \text{SEM}_{\pm} \times t(0.05, \text{error df}) \times \sqrt{2}$. Coefficient of variation (CV%) was calculated as the ratio of the square root of the error mean square to the grand mean. Pearson correlation analysis was used to examine relationships among growth, yield and nutrient-use-efficiency parameters.

3. Results

3.1 Growth attributes and phenology

Plant height at the R4 stage, leaf area index (LAI) at VT and the SPAD chlorophyll index at VT were all significantly ($P \leq 0.01$) influenced by hybrid maturity group, nitrogen environment and nano-bios-

timulant application (Table 3, Fig. 1). Plant height increased progressively with hybrid duration, from a mean of 168.8 cm in H_1 to 187.9 cm in H_2 and 202.3 cm in H_3 , consistent with the longer vegetative phase and greater internode number of longer-duration hybrids. Recommended-N plots were significantly taller (191.5 cm) than low-N plots (181.2 cm), and nano-biostimulant application increased mean plant height from 179.1 cm (control) to 193.6 cm — an effect attributable to the auxin- and cytokinin-like activity of the seaweed-extract component together with improved nutrient acquisition mediated by PGPR and humic acid (Battacharyya *et al.*, 2015; Nardi *et al.*, 2021) [6, 9]. LAI at VT increased from 3.57 (control) to 4.05 (nano-BS; $\text{CD} = 0.41$ at $P = 0.05$), and SPAD index rose from 44.8 to 50.1 over the same comparison, indicating a genuine improvement in canopy photosynthetic capacity rather than a response driven solely by improved assimilate partitioning.

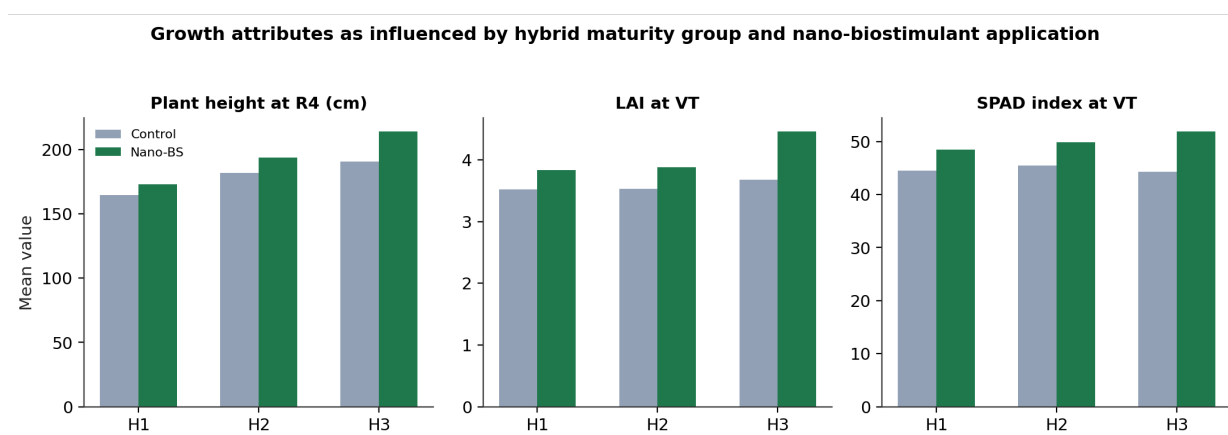


Figure 1. Plant height, leaf area index (LAI) and SPAD chlorophyll index as influenced by hybrid maturity group and nano-biostimulant application (means across nitrogen environments).

Days to 50% tasseling, 50% silking and physiological maturity were governed overwhelmingly by hybrid maturity group, with H_1 , H_2 and H_3 reaching physiological maturity at a mean of 83.9, 100.0 and 121.9 days after sowing, respectively (Table 4, Fig. 2). Nano-biostimulant application did not significantly alter the calendar duration to

physiological maturity within any hybrid (control mean 102.2 days vs. nano-biostimulant mean 101.7 days), confirming that yield and growth benefits arose from enhanced physiological efficiency within an unchanged phenological window, an important consideration for compatibility with existing cropping calendars.

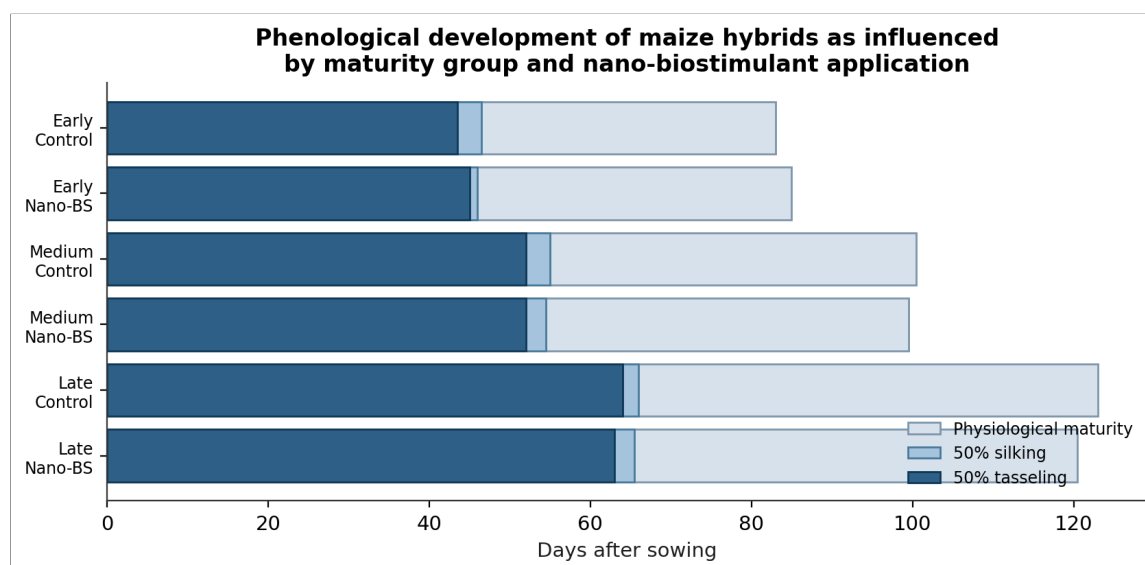


Figure 2. Phenological development (days to 50% tasseling, 50% silking and physiological maturity) of maize hybrids as influenced by maturity group and nano-biostimulant application.

3.2 Yield attributes and grain yield

ANOVA indicated highly significant ($P \leq 0.01$) main effects of hybrid maturity group, nitrogen environment and biostimulant treatment on grain yield, with significant hybrid $\times$ N-environment and hybrid $\times$ biostimulant interactions ($P \leq 0.05$) and a non-significant three-way interaction (Table 5). Grain yield increased progressively with hybrid maturity duration, from 3973 kg ha⁻¹ (H₁) to 4942 kg ha⁻¹ (H₂) and 5936 kg ha⁻¹ (H₃) — a 49.4% improvement of H₃ over H₁ — consistent with the greater total N accumulation and post-silking remobilization capacity of longer-duration hybrids (Bender *et al.*, 2013) [3].

Recommended-N plots yielded 5770 kg ha⁻¹ against 4130 kg ha⁻¹ under low-N (+39.7%), and nano-biostimulant application raised mean grain yield from 4545 kg ha⁻¹ (control) to 5356 kg ha⁻¹ (+17.8%) — a response broadly comparable to, though more conservative than, the 14.8% gain reported for seaweed extract alone by Shukla *et al.* (2019) [7], consistent with the expectation that the additional PGPR and humic-acid components would compound the single-component response.

Table 3. Plant height, leaf area index (LAI) and SPAD chlorophyll index as influenced by hybrid maturity, N-environment and nano-biostimulant consortium.

Treatment	Plant ht. R4 (cm)	LAI (VT)	SPAD (VT)
T ₁ Early Low N Control	164.1	3.39	43.9
T ₂ Early Low N Nano-BS	168.8	3.51	47.6
T ₃ Early Rec. N Control	165.1	3.65	45.1
T ₄ Early Rec. N Nano-BS	177.3	4.15	49.4
T ₅ Medium Low N Control	177.5	3.47	44.6
T ₆ Medium Low N Nano-BS	190.4	3.91	49.2
T ₇ Medium Rec. N Control	186.5	3.59	46.4
T ₈ Medium Rec. N Nano-BS	197.3	3.85	50.5
T ₉ Late Low N Control	182.9	3.48	43.8
T ₁₀ Late Low N Nano-BS	203.5	4.56	50.7
T ₁₁ Late Rec. N Control	198.4	3.87	44.7
T ₁₂ Late Rec. N Nano-BS	224.3	4.35	53.0
General mean	186.3	3.81	47.4
SEm±	7.13	0.141	1.29
CD (P = 0.05)	20.92	0.413	3.79
CV (%)	6.63	6.40	4.71

Table 4. Analysis of variance (F-test significance) for grain yield of maize.

Source of variation	df	Significance
Replication (Block)	2	NS
Hybrid Maturity Group (A)	2	**
Nitrogen Environment (B)	1	**
Biostimulant (C)	1	**
A $\times$ B	2	*
A $\times$ C	2	*

Source of variation	df	Significance
B $\times$ C	1	NS
A $\times$ B $\times$ C	2	NS

The highest grain yield (7724 kg ha⁻¹) was recorded under the late-maturing hybrid with recommended N and the nano-biostimulant consortium (T₁₂) - a 145.3% improvement over the lowest-yielding treatment (T₁: early-maturing, low-N, control; 3149 kg ha⁻¹) (Fig. 3). The significant hybrid $\times$ biostimulant interaction indicated that the absolute biostimulant-mediated yield gain was greatest in the late-matur

ing hybrid (+1449 kg ha⁻¹ under recommended N, $T_{11} \rightarrow T_{12}$) relative to the early-maturing hybrid (+946 kg ha⁻¹, $T_3 \rightarrow T_4$), supporting the hypothesis that hybrids with a longer post-silking remobilization win-

dow derive proportionally greater benefit from biostimulant-enhanced nutrient acquisition (Ciampitti *et al.*, 2012) [4].

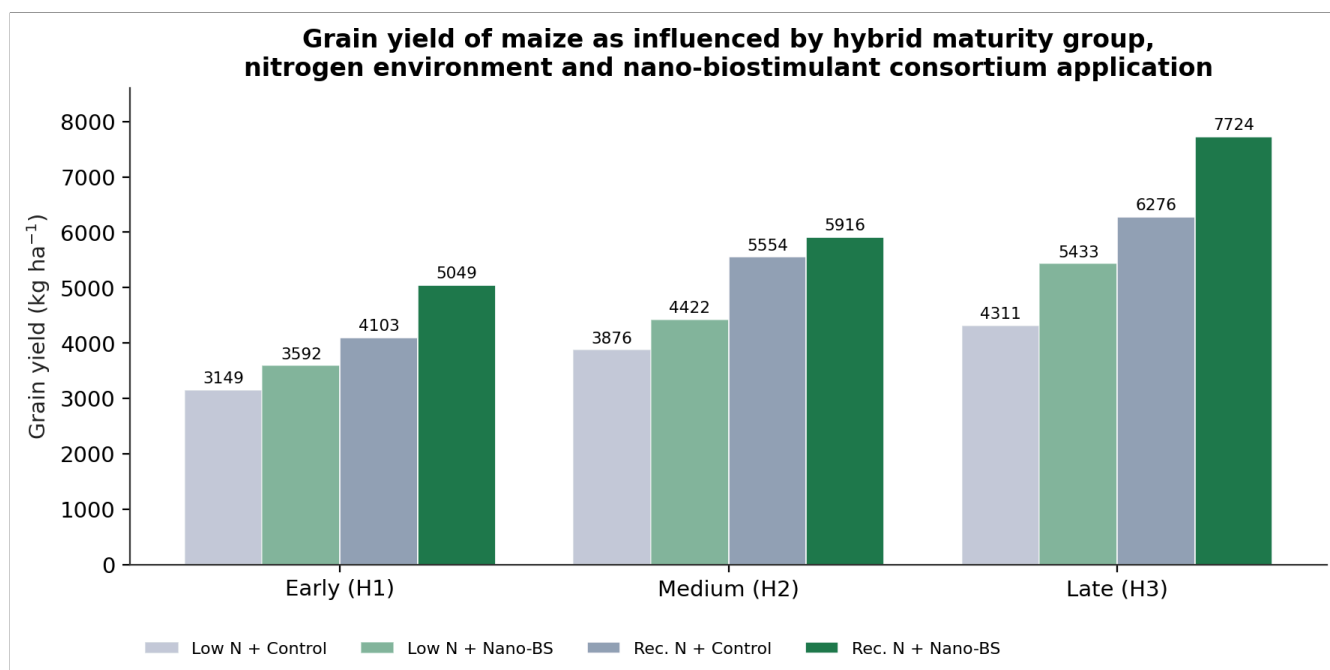


Figure 3. Grain yield of maize as influenced by hybrid maturity group, nitrogen environment and nano-biostimulant consortium application (kg ha⁻¹).

Cobs plant⁻¹ was unaffected by any factor (grand mean 1.04), reflecting the single-eared nature of the hybrids at the plant density used, whereas cob length, kernel rows cob⁻¹, kernels row⁻¹ and 100-kernel weight were all significantly enhanced by hybrid maturity, recommended-N and nano-biostimulant treatment (Fig. 4). The 100-kernel

weight rose from 27.40 g (control) to 29.92 g with nano-biostimulant application (CD = 2.39 g at $P = 0.05$), indicating that sustained nutrient availability translated into more complete grain filling rather than a greater number of florets initiated.

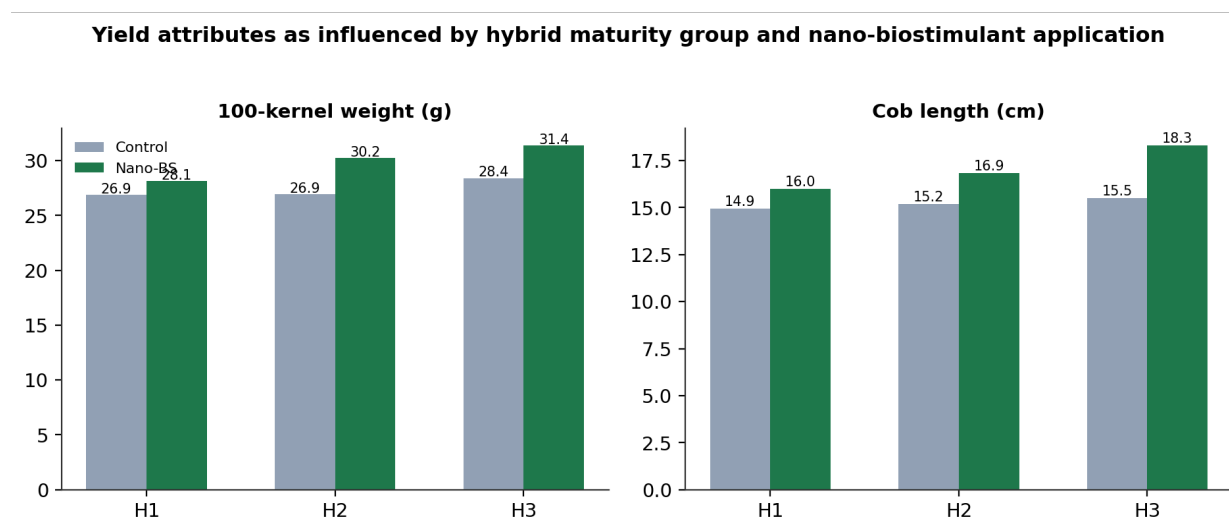


Figure 4. 100-kernel weight and cob length as influenced by hybrid maturity group and nano-biostimulant consortium application.

3.3 Grain quality: protein content

Grain protein content responded most strongly to nitrogen environment, rising from 9.32% under low-N to 9.78% under recommended-N (CD = 0.635% at $P = 0.05$), reflecting the direct structural role of N in storage-protein deposition. Nano-biostimulant application produced a smaller but consistent increase (9.45% to 9.66%), attributable to enhanced N uptake and assimilation via PGPR-mediated biological N fixation and phosphate solubilization acting in concert with improved root proliferation. Hybrid maturity group had comparatively little effect on grain protein percentage, consistent with a dilution effect of higher starch deposition in the larger kernels of longer-dura-

tion hybrids approximately offsetting their greater total N uptake (Fig. 5).

3.4 Soil mineral nitrogen and microbial biomass carbon

Soil NH₄⁺-N at harvest was significantly higher under recommended-N (12.10 mg kg⁻¹) than low-N (9.42 mg kg⁻¹), while nano-biostimulant application produced only a modest increase (10.56 to 10.96 mg kg⁻¹), consistent with more complete crop uptake of applied and mineralized N under biostimulant treatment rather than residual accumulation. Soil available P and K at harvest were both significantly higher in nano-biostimulant-treated plots (P: 20.88 to 23.33 kg ha⁻¹; K: 229.0 to 242.8 kg ha⁻¹), attributable to PGPR-mediated phosphate

solubilization and the rhizosphere-priming action of humic acid (Bashan *et al.*, 2014; Nardi *et al.*, 2021) [8, 9]. Microbial biomass carbon (MBC) increased substantially with nano-biostimulant applica-

tion, from 158.3 to 171.7 mg kg⁻¹ (CD = 31.1 mg kg⁻¹ at $P = 0.05$), directly corroborating the intended rhizosphere-stimulating mode of action of the PGPR component (Fig. 6).

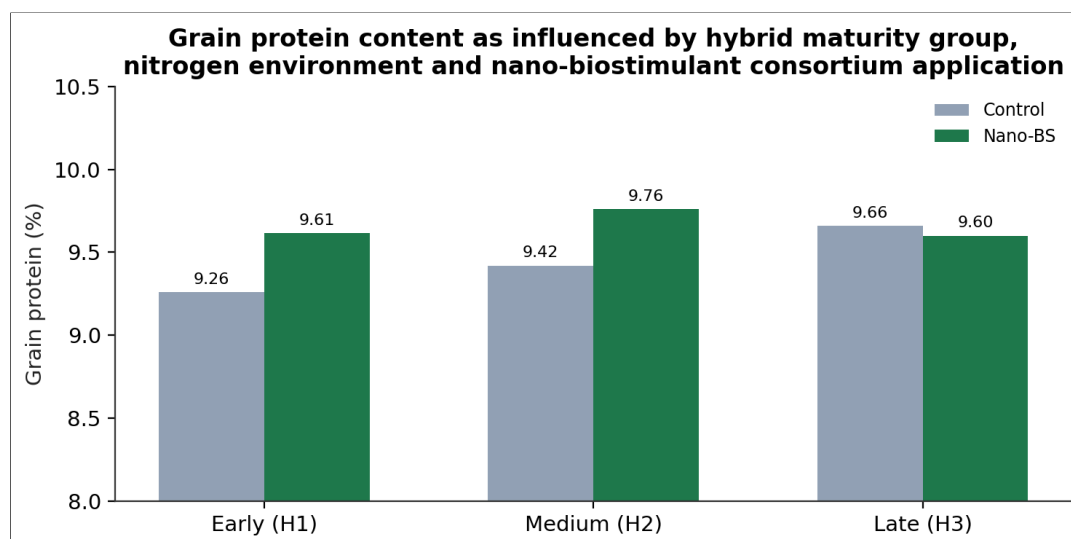


Figure 5. Grain protein content as influenced by hybrid maturity group and nano-biostimulant consortium application.

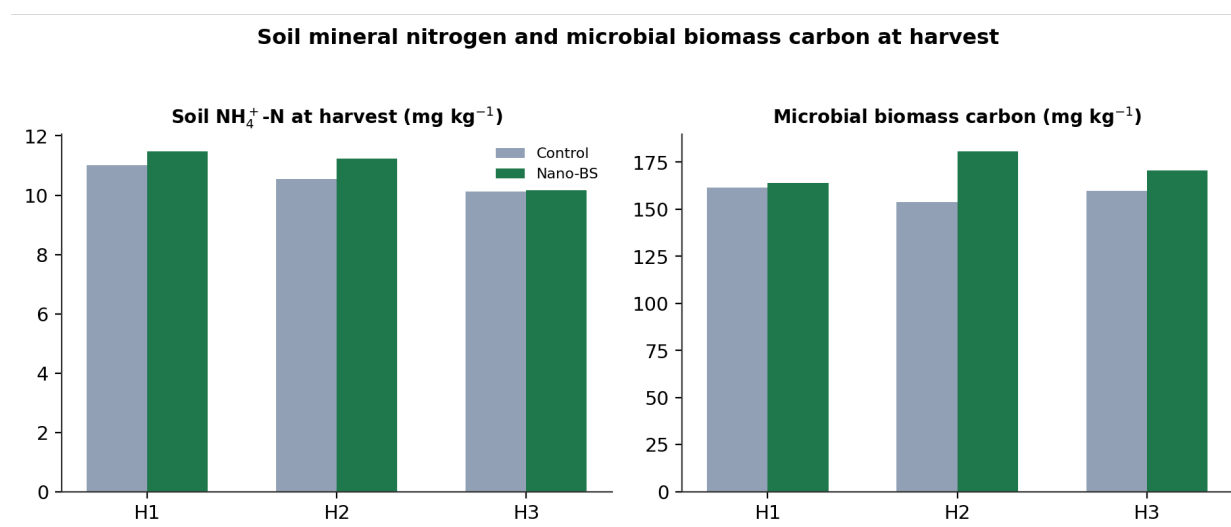


Figure 6. Soil ammoniacal nitrogen and microbial biomass carbon at harvest as influenced by hybrid maturity group and nano-biostimulant application.

3.5 Multi-nutrient-use efficiency (PFPN, NHI, PUE, KUE)

Partial factor productivity of nitrogen (PFPN) was, as expected on a per-kg-applied-N basis, higher under low-N (68.84 kg kg⁻¹) than recommended-N (48.09 kg kg⁻¹). Nano-biostimulant application significantly raised PFPN from 53.62 to 63.31 kg kg⁻¹ (CD = 7.24 at $P = 0.05$), and PFPN increased progressively with hybrid maturity, from

47.15 (H₁) to 58.47 (H₂) and 69.77 kg kg⁻¹ (H₃). Nitrogen harvest index, phosphorus-use efficiency and potassium-use efficiency were likewise all significantly improved by nano-biostimulant application (NHI: 63.49 to 65.30%; PUE: 75.75 to 89.27 kg kg⁻¹; KUE: 113.62 to 133.90 kg kg⁻¹) and increased progressively with hybrid maturity duration, being consistently highest in H₃ (Table 5, Fig. 7).

Table 5. Multi-nutrient-use efficiency indices under recommended nitrogen, by hybrid maturity group and biostimulant treatment.

Treatment	PFPN (kg kg ⁻¹ N)	NHI (%)	PUE (kg kg ⁻¹ P)	KUE (kg kg ⁻¹ K)
H ₁ Rec. N Control	34.19	61.64	68.38	102.58
H ₁ Rec. N Nano-BS	42.07	65.98	84.15	126.23
H ₂ Rec. N Control	46.29	66.96	92.57	138.86
H ₂ Rec. N Nano-BS	49.30	64.50	98.60	147.90
H ₃ Rec. N Control	52.30	64.36	104.59	156.89
H ₃ Rec. N Nano-BS	64.37	67.31	128.74	193.11
General mean (all 12 trt.)	58.46	64.39	82.51	123.76
CD ($P = 0.05$)	7.238	5.255	9.713	14.570

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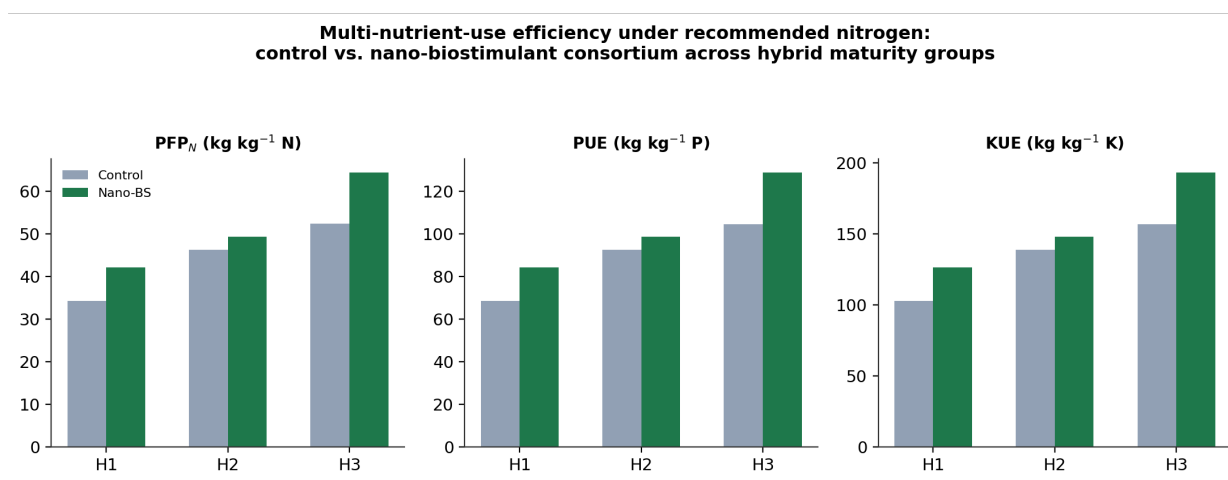


Figure 7. Multi-nutrient-use efficiency (PFPN, PUE, KUE) under recommended nitrogen: control vs. nano-biostimulant consortium across hybrid maturity groups.

3.6 Integrated ANOVA summary

A consistent pattern emerged across all growth, yield, quality, soil and nutrient-use-efficiency parameters examined (Table 6): main effects of hybrid maturity group, nitrogen environment and biostimulant treatment were significant for virtually all traits; the hybrid $\times$ biostimulant interaction was significant specifically for grain yield and for

the yield-linked efficiency indices (PFPN, PUE, KUE); and the three-way interaction remained non-significant throughout. This pattern indicates that the nano-biostimulant consortium acted through a general, physiologically robust mechanism — enhanced root proliferation, rhizosphere microbial activity and canopy photosynthetic capacity — whose absolute benefit scaled with hybrid phenological duration rather than depending on a specific nitrogen environment.

Table 6. Summary of ANOVA significance (*F*-test) for major growth, yield, quality, soil and nutrient-use-efficiency parameters.

Parameter	Hybrid (A)	N-Env (B)	Biostim. (C)	A $\times$ B	A $\times$ C	A $\times$ B $\times$ C
Grain yield	**	**	**	*	*	NS
Plant height	**	*	**	NS	NS	NS
LAI (VT)	**	*	**	NS	NS	NS
100-kernel weight	**	NS	**	*	NS	NS
Grain protein content	NS	**	NS	NS	NS	NS
Soil NH ₄ ⁺ -N	**	**	NS	NS	NS	NS
Microbial biomass C	NS	*	*	NS	NS	NS
PFPN	**	**	**	NS	*	NS
PUE	**	**	**	*	*	NS
KUE	**	**	**	*	*	NS

4. Discussion

The results provide consistent evidence that a phenology-synchronized, chitosan-nanoencapsulated seaweed extract-PGPR-humic acid consortium improves maize growth, yield and multi-nutrient-use efficiency, and that the magnitude of this improvement scales positively with hybrid maturity duration. The combined application of the nano-consortium with recommended nitrogen in the late-maturing hybrid (*T*₁₂) produced the highest values across virtually every growth, yield, quality, soil-biological and nutrient-use-efficiency parameter measured, while the unfertilized, non-biostimulant early-maturing hybrid under low-N (*T*₁) recorded the lowest values for nearly every trait - a coherent hierarchy across independently measured trait categories that strengthens confidence in the underlying biological mechanism rather than reflecting a single confounded observation.

Mechanistically, the parallel enhancement of leaf area index and SPAD chlorophyll index (source capacity), soil available P and K and microbial biomass carbon (rhizosphere nutrient mobilization), and kernel number and kernel weight (sink capacity and grain filling) is consistent with the three complementary modes of action built into the consortium: seaweed-extract-mediated hormonal stimulation of shoot and root growth (Battacharyya *et al.*, 2015) [6], PGPR-mediated biological nitrogen fixation and phosphate solubilization (Bashan *et al.*, 2014) [8], and humic-acid-mediated improvement of root architec-

ture and nutrient transport (Nardi *et al.*, 2021) [9]. Because nano-biostimulant application did not alter the calendar duration to physiological maturity, the yield gains documented here reflect genuine improvement in physiological efficiency within an unchanged phenological window rather than an extension of the growing season, an important practical distinction because it indicates the technology can be adopted without disrupting existing cropping calendars or the scheduling of succeeding crops.

The significant hybrid $\times$ biostimulant interaction for grain yield and for PFPN, PUE and KUE - but not for grain protein, plant height or LAI individually - suggests that the yield-scaling effect of hybrid duration operates principally through the capacity of longer-duration hybrids to translate biostimulant-enhanced nutrient acquisition into a longer post-silking remobilization window, consistent with the physiological framework proposed by Ciampitti *et al.* (2012) [4] and the empirical nutrient-partitioning data of Bender *et al.* (2013) [3]. From an applied standpoint, these findings imply that the economic and environmental returns to phenology-synchronized nano-biostimulant technology are likely to be most pronounced in medium- and, especially, late-maturing hybrid systems managed under recommended nitrogen, where the extended grain-filling period provides the greatest physiological opportunity for biostimulant-mediated nutrient capture - a conclusion with direct relevance to sustainable-intensification strategies that seek to increase productivity while reducing the envi-

ronmental footprint of nitrogen fertilization (Chen *et al.*, 2015; Fixen *et al.*, 2015) [12, 13].

5. Conclusion

Phenology-synchronized application of a chitosan-nanoencapsulated seaweed extract-PGPR-humic acid consortium significantly improved grain yield, growth attributes, yield components, grain protein content, soil biological activity and multi-nutrient-use efficiency of maize across three contrasting hybrid maturity groups and two nitrogen environments. The magnitude of the biostimulant-mediated improvement in grain yield and in PFPN, PUE and KUE was significantly greater in longer-duration hybrids, confirming that late-maturing hybrids possess a greater physiological capacity to convert biostimulant-enhanced nutrient acquisition into recoverable yield and efficiency gains. The combination of a late-maturing hybrid, recommended nitrogen (120 kg N ha⁻¹) and the phenology-synchronized nano-biostimulant consortium produced the highest overall performance among all treatments evaluated and is recommended as the most agronomically favourable combination identified in this study. Because nano-biostimulant application did not delay physiological maturity, this technology can be integrated into existing cropping calendars without disrupting succeeding-crop scheduling. The present findings derive from a single season at one location; multi-season, multi-location validation, together with economic analysis of adoption and mechanistic studies at the molecular level (e.g., nutrient-transporter gene expression across maturity groups), are recommended before extending generalized recommendations to farmers.

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How to cite this article: Rao BA, Babu CS. Phenology-synchronized nano-biostimulant consortia enhance productivity and multi-nutrient-use efficiency of early-, medium and late-maturing maize (*Zea mays* L.) hybrids under contrasting nitrogen environments. *International Journal of Advanced Biochemistry Research*. 2026;SP-10(9):2308-2315. doi: <https://www.doi.org/10.33545/26174693.2026.v10.i9Sac.10168>