J. Trop. Agric. and Fd. Sc. 53 (2)(2025): 17 – 26

Physiological responses of two sorghum genotypes to varying nitrogen level in BRIS soil

Syazrul Iqmal, J[1*]., Nurhazwani, M[1]., Rosnani, A. G[2]., Nurul Afza, K[2]., Mohd Hazrul, Z. T[1]. and Ahmad Ariff Luqman, A. H[1].

[1] *Industrial Crop Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia*
[2] *Industrial Crop Research Centre, MARDI Bachok, 16310 Bachok, Kelantan, Malaysia*

Abstract

Sorghum is a globally significant cereal crop known for its resilience to abiotic stress, making it well-suited for cultivation in marginal environments such as Beach Ridges Interspersed with Swales (BRIS) soil. However, the effects of nitrogen application on its physiological performance in such conditions remain underexplored. This study evaluates the physiological responses of two inbred sorghum genotypes, V1 (India) and V2 (Brachy Sorgho), under three nitrogen application rates: 0 g N/plant (control), 2 g N/plant, and 4 g N/plant, applied at 30 days after sowing (DAS). The experiment followed a factorial 2 × 3 arrangement in a Randomised Complete Block Design (RCBD) with four replications, and the data were analysed using two-way analysis of variance (ANOVA). Key physiological parameters measured included photosynthetic rate, transpiration rate, stomatal conductance, stomatal density, photosynthetic pigments and leaf colour. While nitrogen application did not significantly affect photosynthetic or transpiration rates ($p > 0.05$), possibly due to environmental limitations such as low water holding capacity and soil nutrient retention in BRIS soils, stomatal conductance, stomatal density, pigment concentration, and leaf colour improved significantly ($p < 0.05$) under 2 g N/plant and 4 g N/plant applications. Notably, applying 2 g N/plant nitrogen at 30 DAS optimised physiological responses, resulting in higher grain yield both in terms of panicle grain weight and grain weight/panicle. This application rate also proved most cost-effective, minimising fertiliser input while maintaining productivity. These findings highlight the importance of precise nitrogen management to improve sorghum performance in BRIS soils.

Keywords: *sorghum, physiological response, nitrogen level, photosynthesis efficiency, chlorophyll synthesis*

Introduction

Sorghum (*Sorghum bicolour* L.) is an indigenous African crop and the world's fifth most important cereal grain (Espitia-Hernández et al. 2022; Kazungu et al. 2023). It is cultivated extensively in several countries, including the United States, Nigeria, India, China, Mexico and Australia (Espitia-Hernández et al. 2022). Sorghum consumption has increased significantly in Africa and Asia, driven by its growing role as a staple food, providing approximately 70% of the daily caloric intake in these regions (Tenywa et al. 2018; Thilakarathna et al. 2022). According to FAOSTAT data, global sorghum production in 2022 totaled approximately 57.6 million tonnes, with Nigeria (~6.81 mt), Sudan (~5.25 mt), the United States (~4.77 mt), and Mexico (~4.75 mt) among the leading producers. Africa accounted for just over 51 % of the total global production. Sorghum belongs to the Poaceae family (Sanjana 2017) and is well recognised for its ability to thrive in semi-arid to arid environments, making it a vital crop in regions with limited water availability (Espitia-Hernández et al. 2022). Its deep root system enhances drought tolerance by accessing water stored in deeper soil layers, providing a significant advantage over other cereal crops (Sarshad et al. 2021). The primary agricultural product is sorghum grain, which develops in clusters known as panicles, with each seed enclosed in a hull that varies in colour; white, yellow, red, or brown, depending on the variety (Martiwi et al. 2020; Mustaffer et al. 2024). Globally, different sorghum cultivars including

Article history
Received: 16.06.2025
Accepted: 05.10.2025

Author's full names: Syazrul Iqmal Jalani, Nurhazwani Mustaffer, Rosnani Abd Ghani, Nurul Afza Karim, Ahmad Ariff Luqman Abd Hamid, Mohd Hazrul Zairi Zahid
Corresponding author: syazrul@mardi.gov.my

grain sorghum, sweet sorghum, and forage sorghum, are cultivated for diverse purposes, ranging from human consumption to livestock feed (Hariprasanna & Rakshit, 2016).

Soil nutrient scarcity has been increasingly reported worldwide due to excessive agricultural land use and excessive nitrogen application can lead to soil acidification and degradation of the soil environment, ultimately negatively impacting crop growth and yield (Sun et al. 2020). BRIS soil, a type of sandy soil extensively distributed along the eastern coast of Peninsular Malaysia presents significant challenges for crop production due to its inherently low fertility, poor water-holding capacity, and high sand content (Razali & Salmizi, 2023). These characteristics result in rapid nutrient leaching, limiting plant growth and yield potential. In such nutrient-deficient conditions, optimising nitrogen management is critical for enhancing crop performance. As reported by Ismail et al. (2011), the highly sandy texture, low cation exchange capacity, and strong acidity of Spodosols in BRIS regions significantly reduce soil fertility, necessitating proper fertilization strategies to support successful crop production.

Nitrogen is a vital nutrient for crop production and is primarily absorbed by plants in the forms of nitrate (NO_3^-) and ammonium (NH_4^+). It plays a direct role in plant physiological mechanisms, significantly influencing physiological performance. Nitrogen influences a wide range of physiological processes directly affecting photosynthesis and photosynthetic pigments (Peng et al. 2021). These effects can vary among sorghum genotypes, including chlorophyll production, enzyme synthesis, antioxidative metabolism, and leaf physiological traits. Deficiency leads to reduced chlorophyll and enzyme content, impaired gas exchange, and lower net photosynthetic rates (Ahmad et al. 2022).

Sorghum is a four-carbon (C_4) compound plant that utilises the C_4 photosynthetic pathway, which enhances carbon fixation and reduces photorespiration. Nitrogen deficiency has been shown to impair chloroplast development, reduce the size of photosynthetic cells, result in thinner leaves, decrease stomatal density and disrupt nitrogen allocation, light utilisation, and carbon dioxide (CO_2) assimilation (Makino & Ueno, 2018). As a key component of chlorophyll synthesis, nitrogen plays a crucial role in the light-harvesting capacity of plants. Insufficient nitrogen availability has been reported to negatively impact growth performance and physiological responses in rice, ultimately leading to yield reduction (Peng et al. 2021).

Optimising nitrogen management is essential for maximising sorghum productivity, particularly in nutrient-deficient soils such as BRIS soil. For instance, studies in maize have shown that lower nitrogen supply reduces both the maximum SPAD value ($SPAD_{max}$) and the duration of high-SPAD readings, particularly in lower canopy leaves, indicating diminished chlorophyll retention and photosynthetic longevity in N-deficient plants (Li et al. 2022). This emphasises the critical role of precision in nitrogen management is especially low-buffering sandy soils, where susceptibility to nitrate leaching is high. Under such conditions, splitting nitrogen applications and the use of controlled-release formulations or stabilised urea can enable synchronisation of nitrogen release with plant uptake, thereby curbing environmental losses and improving returns (Qi et al. 2021).

Nitrogen is a key driver of photosynthetic efficiency, as it enhances chlorophyll synthesis and improves light energy capture. As a fundamental component of chlorophyll, amino acids, nucleic acids, and enzymes, nitrogen plays a central role in regulating photosynthesis, protein synthesis, and energy metabolism (Ahmad et al. 2022). Moreover, nitrogen regulates stomatal function, balancing gas exchange and water-use efficiency to sustain photosynthesis under variable conditions. It also influences the synthesis of photosynthetic pigments, such as chlorophyll and carotenoids, which are vital for plant growth and stress resilience. Therefore, this study aims to determine the optimal nitrogen application rate for improving the physiological responses of selected sorghum genotypes. By identifying the most effective nitrogen level, this research seeks to enhance sorghum's adaptive potential, ensuring better growth, higher yield, and improved resource use efficiency in challenging growing conditions.

Materials and method

Plant material and growth condition

The planting materials used in this study comprised two genotypes: V1 (India) and V2 (Brachy Sorgho), which are inbred varieties derived from commercial seeds in India. An adaptability study was conducted on these genotypes, and they exhibited optimal growth performance and high yields, leading to their selection for further evaluation to determine the optimal nitrogen requirements in 2024 (Mustaffer et al. 2024). The trial plot was located at Bachok, Kelantan, Malaysia (3.890981, 100.858494) in BRIS soil and the evaluation was conducted from February until August 2024. Selected genotypes were grown in a plot area of 25 m^2 (5 m × 5 m) with four replications. Land preparation was done using mechanisation and raised beds, while the organic matter was spread manually (6 t/ha) a week before planting. The planting system used 75 cm × 10 cm (within row) plant spacing and a plant population of approximately 133,333 plants/ha.

Fertiliser application

A compound fertiliser containing nitrogen (N), phosphorus (P) and potassium (K) (NPK 15:15:15) was uniformly applied at a rate of 800 kg/ha on the sowing day. The fertiliser was placed adjacent to the sowing hole (5 cm between holes) at a depth of 2 cm. Nitrogen application, in the form of urea (46% N), was applied once at 30 days after sowing (DAS) at three different application rates: 0 g N/plant (control), 2 g N/plant (266.67 kg/ha), and 4 g N/plant (533.33 kg/ha).

The chosen nitrogen rates were based on previous research conducted by Holman et al. (2024), who conducted a multi-location trial in Kansas, USA, and reported that urea-N application rates of 134 – 160 kg N/ha (equivalent to ~290 – 348 kg urea/ha) were tested on grain sorghum and grain corn as a baseline for N sources. A significant increase in corn grain yield was observed with the use of enhanced nitrogen sources. The application rates used in this study (2 – 4 g/plant) fall within and slightly exceed this agronomic range, thereby allowing the assessment of crop response under both optimal and high-input nitrogen regimes.

Photosynthesis efficiency

Gas exchange parameters, such as photosynthetic rate, transpiration rate and stomatal conductance, were sampled from three plants per experimental unit and recorded between 10:00 a.m. and 12:00 a.m. on the fully expanded third leaf from the top with an LI-6800 portable photosynthesis system (LI-COR, Biosciences, Lincoln, NE, United States) at 42 DAS. The selection of 42 DAS for measuring photosynthetic efficiency in sorghum is justified by its alignment with the flag leaf emergence stage, typically occurring between 41–50 DAS. This stage represents a critical phase in crop development, during which leaf photosynthetic capacity significantly contributes to biomass accumulation and grain formation (Piccinni et al. 2009). During the measurement period, the photosynthetic photon flux density (PPFD) was controlled at 1,500 μmol/m^2/s, the block temperature was set at 30 °C, the carbon dioxide concentration in the air entering the leaf chamber was maintained at 400 μmol/mol, and the relative humidity was at 50 – 70%. Each treatment was replicated three times.

Stomatal density was assessed using a destructive leaf impression technique adapted for field and laboratory evaluation. Fully expanded, sun-exposed leaves from each treatment were selected for analysis, and impressions were made on the adaxial (upper) surface, approximately at the middle of the flag leaf emergence (avoiding the midrib) to standardise sampling location and minimise anatomical variability.

A thin, even layer of clear nail polish was applied (3 cm length) directly onto the adaxial leaf surface using a fine brush and allowed to air-dry completely (10 – 15 minutes), forming a thin film that accurately captured epidermal features. Once dried, a piece of transparent tape was gently pressed onto the dried nail polish film to lift the impression. The tape containing the epidermal imprint was carefully removed and mounted onto a clean glass microscope slide for microscopic observation.

Microscopic analysis was performed using a binocular compound light microscope at 60 X magnification. Stomata were counted in three to five randomly selected non-overlapping fields of view/leaf sample to account for intra-leaf variability and ensure representative sampling. The field of view area was determined using a calibrated micrometer scale, and stomatal density was expressed as the number of stomata per mm^2 using to the following formula: Stomatal density was calculated as the average number of stomata/field of view, divided by the area of the field of view (stomata/mm^2).

Photosynthetic pigment

The same leaf was measured for photosynthesis efficiency to determine the content of photosynthetic pigments, including chlorophyll a, chlorophyll b, carotenoids, and relative chlorophyll content. Six leaf discs of leaf samples (6 mm of each disc from the middle of a leaf) were collected and immersed in dimethyl sulfoxide (DMSO) at 65˚C for four hours until the leaf tissue became colourless. The photosynthetic pigments were quantified using a spectrophotometer at multiple wavelengths (649, 665, 480 and 510) and calculated as described by Hiscox and Israelstam (1979).

Leaf colour

Leaf samples were collected from healthy, fully expanded leaves at 42 DAS with a uniform vegetative stage. Colour parameters (L*, a*, b*, C, h) were measured using a Minolta CR-300 Chroma meter (Minolta Camera Co., Osaka, Japan) in configured to the Lab* colour space. The chromameter's measuring head was placed on the adaxial (upper) surface of each leaf to record colour values. For each measurement, the device provided L* (lightness), a* (red-green axis) and b* (yellow-blue axis) values. Data was collected on each experimental unit from three leaves (two measurements/leaf), and the measurements were taken from the green spots of the leaves.

Yield component

Five fresh sorghum panicles were harvested and sun-dried until a moisture content of approximately 16% was achieved. Grain weight with panicle (g/plant) and grain weight/panicle (g) were measured from five freshly harvested panicles/plot. Panicles were air-dried for five days, after which the total weight of each panicle including grains was recorded using a digital balance. Grains were then manually separated and weighed individually to obtain the final values for grain weight/panicle.

Experimental design and statistical analysis

The experiment was conducted using a factorial Randomised Complete Block Design (RCBD) with four replications. The first factor was genotype, consisting of two inbred sorghum varieties: V1 (India) and V2 (Brachy Sorgho). The second factor was nitrogen application rate, with three levels: 0 g N/plant (control), 2 g N/plant, and 4 g N/plant. Nitrogen was applied at 30 DAS.

Standard agronomic practices were uniformly applied across all treatment plots. For uniformity assessment of plant population, data were collected from five randomly selected plants/experimental unit. Collected data were normally distributed and subjected to two-way analysis of variance (ANOVA) using SAS software (version 9.4) to determine the main effects and interactions between genotype and nitrogen levels. Mean comparisons were performed using Duncan's Multiple Range Test (DMRT) at a significance level of $p \leq 0.05$.

Results and discussion

Photosynthesis efficiency

The study was conducted to investigate the effects of different nitrogen rates on photosynthesis efficiency in two sorghum genotypes, focusing on photosynthesis rate, transpiration rate, stomatal conductance, and stomatal density (*Tables 1* and *2*). Photosynthesis and transpiration rates did not respond significantly ($p < 0.45$ and $p < 0.19$, respectively) to nitrogen application at 42 days, indicating that within the tested levels, nitrogen availability did not limit photosynthesis in these sorghum genotypes.

Photosynthesis and transpiration rates in sorghum are primarily influenced by environmental conditions rather than nitrogen application. The transpiration process is highly responsive to microclimatic factors such as temperature and humidity, which regulate plant water loss. The absence of a significant nitrogen effect on transpiration may be attributed to fluctuations in transpiration rates across different nitrogen levels in the evaluated sorghum genotypes. This finding is consistent with Shah et al. (2017), who reported that nitrogen application had no significant effect on transpiration rates in cotton at 40 days after emergence.

The findings (*Table 1*) indicate that nitrogen application significantly affected stomatal conductance ($p < 0.04$), highlighting its critical role in regulating gas exchange and plant water dynamics. The increase in stomatal conductance at 4 g N/plant indicates an enhanced capacity for gas exchange regulation, likely driven by improved leaf nitrogen status. This enhancement may reflect the positive role of nitrogen in stimulating photosynthetic efficiency through its influence on key physiological processes (Shah et al. 2017; Fang et al. 2018). Transpiration rate response to environmental conditions induces changes in the stomatal density of newly developed leaves (Lake & Woodward, 2008). These results demonstrate that applying 2 g N/plant and 4 g N/plant increases the transpiration rate, optimises stomatal conductance and influences the structural characteristics of the leaves in terms of the number of stomata/unit area. Sorghum appears to be resilient in its photosynthetic and transpiration responses under different nitrogen levels, while nitrogen influences stomatal regulation.

Conversely, the lack of significant differences ($p < 0.05$) in stomatal density across treatments (*Table 2*) indicates that nitrogen supplementation did not affect stomatal patterning or development during leaf expansion. This finding suggests that anatomical characteristics such as stomatal density in sorghum are relatively stable and less responsive to nitrogen availability within the tested range. Instead, nitrogen's primary influence was on stomatal conductance, which increased significantly at the highest nitrogen rate (4 g N/plant), implying that physiological regulation of stomatal aperture occurred rather than structural adjustment. These findings align with the conclusions of Ferguson et al. (2024), who demonstrated in C_4 sorghum that reductions in stomatal density did not compromise carbon assimilation, indicating that gas exchange capacity in C_4 species is largely governed by dynamic stomatal aperture control rather than anatomical changes. Therefore, while stomatal density remained unchanged, the observed increase in stomatal conductance with nitrogen application could indirectly enhance photosynthetic efficiency over time or under specific environmental conditions that demand higher gas exchange regulation.

Table 1. Mean square ANOVA of sorghum genotypes subjected to different nitrogen rates at 42 DAS

Sources of variances	Photosynthesis rate (µmol/ms)	Transpiration rate (mmol)	Stomatal conductance (mmol/ms)	Stomatal density/ 5 mm^2
Variety	0.139^{ns}	0.231^{ns}	0.06^{ns}	0.544^{ns}
Nitrogen	0.45^{ns}	0.19^{ns}	0.04*	0.05*
Block	0.05*	0.02*	0.15^{ns}	0.765^{ns}
Variety*nitrogen	0.33^{ns}	0.1^{ns}	0.05*	0.88^{ns}
Grand mean	33.1	2.56	0.176	12.2
Coefficient of variation (C.V.) %	13.69	22.93	32.43	8.08

Note: mean followed by * is significant at $p < 0.05$

Table 2. Effect of different rates of nitrogen on photosynthesis efficiency variables at 42 DAS

Nitrogen rate/plant	Photosynthesis rate (umol/ms)	Transpiration rate (mmol)	Stomatal conductance (mmol/ms)	Stomatal density/ 5 mm^2
0 g	32.18^{a}	2.44^{a}	0.14^{b}	11.87^{b}
2 g	32.34^{a}	2.37^{a}	0.14^{b}	12.25^{a}
4 g	34.74^{a}	2.89^{a}	0.24^{a}	12.5^{a}

Note: Mean values in the same column followed by the same letter are not significantly different at $p < 0.05$

Photosynthetic pigment

Photosynthetic pigments such as chlorophylls and carotenoids are integral to light capture in photosynthesis, as they absorb and channel solar energy into the photosystems, thereby driving primary productivity in plants (Nguyen & Sung, 2025). Nitrogen, a crucial component of the chlorophyll molecule, directly regulates the synthesis and concentration of photosynthetic pigments, thereby influencing light-harvesting efficiency and overall photosynthetic performance (Peng et al. 2021). Adequate nitrogen supply enhances pigment production, improving photosynthetic efficiency and plant health. However, excessive or insufficient nitrogen can disrupt this balance, leading to reduced photosynthetic capacity. Understanding the relationship between nitrogen application and photosynthetic pigment levels is essential for optimising crop performance, particularly in nutrient-poor soils like BRIS soil.

Evaluation of *Tables 3* and *4* revealed no significant genotypic differences between V1 and V2 in total chlorophyll, chlorophyll a, chlorophyll b, carotenoids and relative chlorophyll content, indicating comparable pigment profiles and similar physiological responses between the two genotypes. Similar findings were reported by Tranavičienė et al. (2007), who observed no significant differences in photosynthetic pigment between the two investigated wheat varieties were observed.

Meanwhile, the result indicates that the nitrogen level has a significant, time-dependent effect on photosynthetic pigment in sorghum. Early in the plant's life cycle (30 DAS), nitrogen levels do not significantly influence overall variables, possibly because nitrogen uptake is initially slower, or the plants have sufficient nitrogen reserves from seed or soil to sustain early growth. Similarly, Tranavičienė et al. (2007) reported that as the plants mature (60 and 90 DAS), nitrogen's effect on overall variables becomes highly significant. This trend is expected, as nitrogen is a crucial component of chlorophyll synthesis, which plays a key role in photosynthesis. Increased chlorophyll content with higher nitrogen levels likely enhances the plant's photosynthetic capacity, contributing to better growth and productivity (Fornari et al. 2020).

Application of nitrogen at 2 g N/plant and 4 g N/plant significantly enhanced total chlorophyll, chlorophyll a, chlorophyll b, carotenoids and relative chlorophyll content in sorghum at later growth stages (60 and 90 DAS), as shown in *Table 5*, underscoring its critical role in sustaining photosynthetic activity as plants mature. Nitrogen is a vital element for sustaining chlorophyll content in plants, which is crucial for maintaining photosynthetic activity (Dovale et al. 2012). These findings highlight the potential benefits of adequate nitrogen fertilisation for maximising chlorophyll content and, consequently, the photosynthetic efficiency of sorghum.

Plants receiving 2 g N/plant and 4 g N/plant exhibited higher total chlorophyll content (49.18 mg/L and 54.49 mg/L, respectively) compared to the control (40.18 mg/L) (*Table 5*). This was further reflected in the significantly greater concentrations of total chlorophyll, chlorophyll *a*, and chlorophyll *b* observed with the 2 g N/plant and 4 g N/plant applications. Higher chlorophyll content enhances the plant's photosynthetic efficiency by facilitating greater light energy capture, thereby improving overall productivity.

An increase in chlorophyll *a* content was observed with nitrogen application as plants treated with 2 g N/plant and 4 g N/plant recorded higher values (40.44 mg/L and 44.97 mg/L, respectively) compared to the control (33.11 mg/L). A similar trend was observed for chlorophyll *b* content, which increased with higher nitrogen levels. Plants receiving 2 g N /plant and 4 g N/plant exhibited significantly greater chlorophyll *b* concentrations (8.74 mg/L and 9.51 mg/L, respectively) than those under the control application (7.07 mg/L), demonstrating a clear positive response to nitrogen application. These findings align with Moeinirad et al. (2021), who reported that increasing nitrogen availability enhances the accumulation of chlorophyll *a* and *b*. Similarly, Peng et al. (2021) observed an upward trend in chlorophyll *a*, chlorophyll *b*, and carotenoid content with increasing nitrogen application rates, further supporting the role of nitrogen in enhancing photosynthetic pigment synthesis.

Carotenoid content followed a similar trend, with 2 g N/plant and 4 g N/plant applications showing significantly higher levels (16.03 and 17.73) than the 0 g application (13.67 mg/L). Carotenoids, which also increase with nitrogen, play a crucial role in protecting chlorophyll from oxidative damage and are involved in light harvesting (Hashimoto et al. 2016). Thus, higher carotenoid levels in the 2 g N/plant and 4 g N/plant applications enhance the plant's resilience to environmental stress, potentially enhancing photosynthetic stability and efficiency.

The relative chlorophyll content was significantly lower in the 0 g application (47.65) compared to the 2 g N/plant application (51.51). However, no significant difference was observed between the 2 g N/plant and 4 g N/plant applications, indicating that the optimum relative chlorophyll content value was reached at around 2 g N/plant of nitrogen. Increasing nitrogen levels to 2 g N/plant and 4 g N/plant led to higher concentrations of these pigments, indicating improved photosynthetic capacity.

These findings indicate that under the given conditions, a nitrogen application rate of 2 g N/plant is optimal for enhancing chlorophyll content in sorghum while maintaining a balance between resource efficiency and physiological benefits. According to Shi et al. (2020), excessive nitrogen disrupts critical biochemical and physiological processes, leading to metabolic imbalances. Similarly, *Britto & Kronzucker* (2002) reported that while low nitrogen levels are beneficial, excessive ammonium can induce toxicity, disrupt nutrient uptake, and alter carbon metabolism, ultimately affecting plant growth and development.

Table 3. Mean square ANOVA for the effect of different nitrogen rates on chlorophyll content and photosynthetic pigment at 30, 60 and 90 DAS

Sources of variances/DAS	Total chlorophyll (mg/L)			Chlorophyll a (mg/L)			Chlorophyll b (mg/L)		
	30	60	90	30	60	90	30	60	90
Variety	0.562^{ns}	0.765^{ns}	0.925^{ns}	0.614^{ns}	0.909^{ns}	0.953^{ns}	0.351^{ns}	0.267^{ns}	0.502^{ns}
Nitrogen	0.430^{ns}	0.001**	0.0006***	0.417^{ns}	0.002**	0.0006***	0.515^{ns}	0.002**	0.001**
Block	0.419^{ns}	0.963^{ns}	0.234^{ns}	0.427^{ns}	0.905^{ns}	0.234^{ns}	0.393^{ns}	0.663^{ns}	0.290^{ns}
Variety *nitrogen	0.380^{ns}	0.547^{ns}	0.204^{ns}	0.38^{ns}	0.436^{ns}	0.204^{ns}	0.336^{ns}	0.996^{ns}	0.296^{ns}
Grand mean	34.51	47.95	37.24	28.26	39.5	29.55	6.24	2.44	7.69
Coefficient of Variation (C.V.) %	31.11	13.56	14.15	31.62	13.86	14.32	29.4	13.88	14.17

Note: Mean followed by * is significant at $p < 0.05$

Table 4. Mean square ANOVA for the effect of different nitrogen rates on photosynthetic pigment at 30, 60 and 90 DAS

Sources of variances/DAS	Caratenoid (mg/L)			Relative chlorophyll content (umol/ms)		
	30	60	90	30	60	90
Variety	0.260^{ns}	0.462^{ns}	0.505^{ns}	0.04*	0.585^{ns}	0.363^{ns}
Nitrogen	0.442^{ns}	0.001**	0.0006***	0.77^{ns}	0.02*	0.410^{ns}
Block	0.205^{ns}	0.666^{ns}	0.445^{ns}	0.456^{ns}	0.735^{ns}	0.384^{ns}
Variety*nitrogen	0.523^{ns}	0.974^{ns}	0.256^{ns}	0.328^{ns}	0.04*	0.194^{ns}
Grand mean	12.18	15.81	12.89	38.42	49.76	38.65
Coefficient of variation (C.V.) %	16.7	11.33	12.43	17.19	4.94	12.55

Note: Mean followed by * is significant at $p < 0.05$

Table 5. Effect of different nitrogen rates on photosynthetic pigment at 60 DAS

Nitrogen rate/plant	Total chlorophyll (mg/L)	Chlorophyll a (mg/L)	Chlorophyll b (mg/L)	Caratenoid (mg/L)	Relative chlorophyll content (umol/ms)
0 g	40.18^{b}	33.11^{ab}	7.07^{b}	13.67^{b}	47.65^{b}
2 g	49.18^{a}	40.44^{a}	5.74^{a}	16.03^{a}	51.51^{a}
4 g	54.49^{a}	44.97^{a}	9.51^{a}	17.73^{a}	50.14^{ab}

Note: Mean values in the same column followed by the same letter are not significantly different at $p < 0.05$

Leaf colour

The results indicate leaf colour changes in response to varying levels of nitrogen application (*Table 6* and *7*). Leaf colour is primarily influenced by the type and concentration of pigments, particularly chlorophyll and carotenoids, which are the two main pigments involved in photosynthesis (Markwell & Namuth, 2003). As nitrogen levels increase, lightness (L*) decreases from 54.50 – 47.99 and the leaves become darker. The decrease in lightness affects the overall brightness of the leaves, potentially being related to the increased chlorophyll content. Xue & Yang (2009) reported that increases in nitrogen levels and leaf colour both enhanced the amount of chlorophyll in the leaves.

The chlorophyll a* value indicates the red-green spectrum (positive = red, negative = green). All application rates showed negative chlorophyll a* values, indicating a dominance of green hues due to the presence of chlorophyll. The control (−17.38) was significantly greener than the nitrogen-treated plants, which had higher (less negative) a* values (−16.09 and −15.83). A reduced green intensity with nitrogen could indicate a shift in the pigment balance due to changes in chlorophyll-to-carotenoid ratio or the onset of senescence onset at higher nitrogen levels (Zhao et al. 2022).

The chlorophyll b* value (blue-yellow spectrum) was significantly higher in the control (33.43) and decreased with nitrogen application (27.76 – 27.79). Higher chlorophyll b* values suggest the presence of yellow tones, often linked with chlorosis or lower chlorophyll density.

Chroma (*C**), representing colour saturation or intensity, declined with nitrogen application (38.01 in control to 31.11 in 4 g N), indicating deeper, less vivid colour. This can be attributed to increased chlorophyll masking other pigments, especially under optimal nitrogen supply (Yuan et al. 2021). Similarly, the hue angle (*h*), which reflects the actual colour tone (measured in degrees), was highest in the control (120.20°), decreasing slightly but significantly with nitrogen addition. Although this shift is minor, the colour moves slightly from a yellow-greenish hue to a less saturated greenish hue with higher nitrogen levels.

Application of nitrogen at either 2 g N/plant or 4 g N/plant resulted in darker, less vivid, and slightly reduced yellow-green leaf colouration. This visual response aligns with the photosynthetic pigment data (*Table 7*), which demonstrates that higher nitrogen rates improve leaf pigmentation, reflecting healthier plant growth. The observed changes in leaf colour are consistent with the higher recorded chlorophyll content recorded at elevated nitrogen rates, further supporting the role of nitrogen in modulating leaf pigmentation and overall photosynthetic efficiency.

Yield component

Nitrogen application affects the yield composition of sorghum, specifically the weight of panicle with grain and the weight of grains per panicle (*Figures 1* and *2*). The differential effects of 2 g N/plant or 4 g N/plant on sorghum's physiological performance and yield indicate the complex interaction between nutrient availability, plant physiological response, and resource allocation in BRIS soil. Nitrogen is essential for enhancing overall plant performance, however excessive application does not necessarily translate into higher yield. This study revealed that although the 4 g N/plant increased chlorophyll content (*Table 5*), it did not significantly enhance photosynthesis rate, transpiration rate (*Table 2*) or improve overall grain yield. Instead, the 2 g N/plant resulted in the highest yield of the total weight of panicle with grain (121.60 ± 24.40 g) and the weight of grains per panicle (94.33 ± 23.61 g) respectively, indicating that the role of nitrogen is not solely determined by chlorophyll content but also by achieving an optimal source–sink balance and accounting for environmental limitations. When source activity exceeds sink demand, carbohydrate accumulation in leaves may trigger feedback inhibition of photosynthesis, a phenomenon exacerbated under nitrogen-deficient conditions (Paul & Driscoll, 1997). Maintaining an optimal source–sink balance is critical for maximizing yield, especially in crops, as it governs how efficiently photosynthates are partitioned among developing plant parts (Paul & Foyer, 2001).

Moreover, excessive nitrogen tends to promote vegetative growth and higher chlorophyll levels at 4 g N /plant did not translate into higher yield of the total weight of panicle with grain (88.58 ± 17.41 g) and the weight of grains per panicle (87.02 ± 4.74 g) respectively, as a greater proportion of assimilates was likely diverted toward vegetative rather than reproductive growth. Excess nitrogen can lead to an imbalance, where the source (leaves) develops excessively, but the assimilates produced are not efficiently translocated to the grains. This result was supported by a field experiment in Iran using four nitrogen rates (0, 80, 160 and 200 kg ha^{-1}), which revealed that increasing nitrogen application with urea reduced sorghum grain yield by 2.04% to 6.58%, indicating no yield benefit from higher nitrogen levels (Soleymani et al. 2011; Fei et al. 2025).

These findings on nitrogen application in sorghum indicate that nitrogen significantly impacts grain production by enhancing physiological performance in BRIS soil. Specifically, the application of 2 g N/plant markedly improved grain yield by optimising key physiological processes related to nitrogen uptake and photosynthetic efficiency. The 2 g N/plant application, on the other hand, maintained an optimal balance between leaf area, chlorophyll content, and grain filling, ensuring efficient conversion of photosynthates into productive biomass. Moreover, optimal nitrogen levels have been shown to sustain prolonged and active photosynthesis, contributing to greater biomass accumulation and increased grain yield in sorghum (Zhao et al. 2005). In addition, Ostmeyer et al. (2022) identified key physiological traits that improve nitrogen use efficiency (NUE), thereby enhancing both grain yield and quality. Collectively, these results suggest that applying 2 g N/plant (266.67 kg/ha) effectively supports critical physiological mechanisms necessary for maximising sorghum grain production.

Table 6. Mean square ANOVA for the effect of different nitrogen rates on colourimetry value at 42 DAS

Sources of variance	L*	a*	b*	C*	h
Variety	0.419^{ns}	0.076^{ns}	0.16^{ns}	0.13^{ns}	0.993^{ns}
Nitrogen	0.0006***	0.013*	0.0007***	0.0007***	0.081^{ns}
Block	0.664^{ns}	0.411^{ns}	0.636^{ns}	0.608^{ns}	0.499^{ns}
Variety*nitrogen	0.381^{ns}	0.39^{ns}	0.222^{ns}	0.241^{ns}	0.711^{ns}
Grand mean	50.59	16.43	24.49	33.96	119.3
Coefficient of variation (C.V.) %	5.44	5.88	9.4	8.3	1.72

Note: mean followed by * is significant at $p < 0.05$

Table 7. Effect of different nitrogen rates on colourimetry (Lab (CIE L*ab** colour space), LCH (lightness, chroma and hue)) at 42 DAS

Nitrogen rate/plant	L*	a*	b*	C*	h
0 g	54.50^{a}	-17.38^{a}	33.43^{a}	38.01^{a}	120.20^{a}
2 g	49.30^{b}	-16.09^{b}	27.76^{b}	32.14^{b}	119.93^{ab}
4 g	47.99^{b}	-15.83^{b}	27.79^{b}	31.11^{b}	117.91^{b}

Note: Mean values in the same column followed by the same letter are not significantly different at $p < 0.05$

Overapplication of nitrogen not only leads to unnecessary input costs but can also reduce grain filling efficiency and contribute to environmental concerns, such as nutrient leaching and soil degradation. From a practical perspective, translating the 2 g N/plant application rate into a field scale is essential for large-scale production planning. In this study, at 2 g N/plant and a density of 133,333 plants/ha, the application rate was 266.7 kg N/ha. This benchmark allows growers to adjust fertilisation plans according to field size, soil fertility status, and economic considerations, ensuring the most cost-effective and environmentally responsible nitrogen use. On a commercial scale, adopting this optimised rate could improve profitability by reducing unnecessary fertiliser costs while maintaining or even enhancing grain yield.

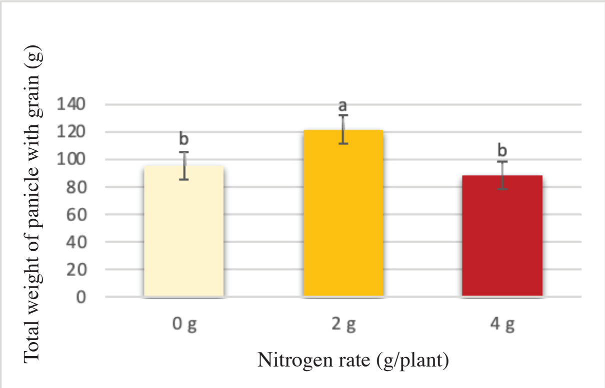

Figure 1. Effect of nitrogen application rate on total panicle weight with grain in sorghum. Different letters above bars indicate significant differences at p <0.05 (DMRT)

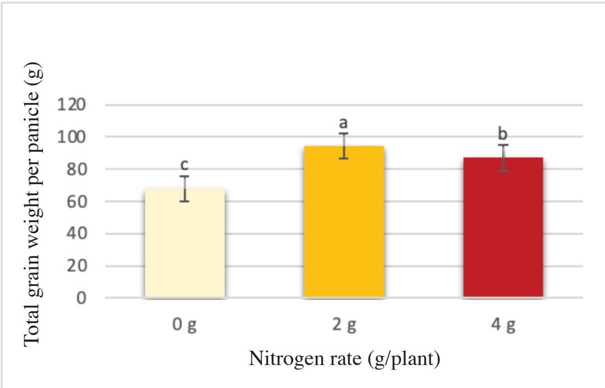

Figure 2. Effect of nitrogen application rate on total grain weight per panicle in sorghum. Different letters above bars indicate significant differences at p <0.05 (DMRT)

Conclusion

This study highlights the significant role of nitrogen application in influencing sorghum's physiological performance, particularly photosynthetic efficiency, pigment concentrations, and leaf colour. Both 2 g N/plant and 4 g N/plant positively impacted these traits, enhancing the plant's physiological performance and grain productivity. However, while 4 g N/plant stimulated excessive vegetative growth, it did not translate into higher grain yield. Instead, the highest grain yield was observed with 2 g N/plant, indicating that beyond a certain threshold, additional nitrogen may contribute more to biomass accumulation than grain production. These findings underscore the importance of optimising nitrogen application rates to achieve a balance between vegetative growth and grain yield. Effective nitrogen management strategies should focus on maximising yield potential while ensuring efficient nutrient use. Overapplication of nitrogen not only leads to unnecessary input costs but can also reduce grain filling efficiency and contribute to environmental concerns, such as nutrient leaching and soil degradation. In conclusion, while both 2 g N/plant and 4 g N/plant improved sorghum performance, the results indicate that careful regulation of nitrogen application is essential for enhancing nitrogen use efficiency, sustain productivity, and prevent the adverse effects of excessive fertilisation. Future research should explore additional factors, such as soil characteristics and environmental conditions, to refine nitrogen recommendations for optimal sorghum production.

Acknowledgement

This research was conducted under the Projek Inisiatif Ekonomi MADANI. The authors sincerely thank the Industrial Crop Research Centre for their support and appreciate everyone who contributed to this study.

References

Ahmad, I., Zhu, G., Zhou, G., Song, X., Hussein Ibrahim, M. E., & Ibrahim Salih, E. G. (2022). Effect of N on Growth, Antioxidant Capacity, and Chlorophyll Content of Sorghum. Agronomy, 12(2), 501.

Britto, D. T., & Kronzucker, H. J. (2002) NH_{4+} toxicity in higher plants: a critical review. *Journal Plant Physiol*. 159, 567 – 584. https://doi.org/10.1078/0176-1617-0774.

Dovale, J. C., Delima, R. O., & Fritsche-Neto, R. (2012). "Breeding for nitrogen use efficiency" in Plant Breeding for Abiotic Stress Tolerance, eds R. Fritsche-Neto and A. Borem (Berlin: Springer), 53 – 65

Espitia-Hernández, P., Chávez González, M. L., Ascacio-Valdés, J. A., Dávila-Medina, D., Flores-Naveda, A., Silva, T., Ruelas Chacón, X., & Sepúlveda, L. (2022). Sorghum (*Sorghum bicolor* L.) as a potential source of bioactive substances and their biological properties. *In Critical Reviews in Food Science and Nutrition*. 62(8), 2269 – 2280.

Fang, X., Li, Y., Nie, J., Wang, C., Huang, K., Zhang, Y., Zhang, Y., She, H., Liu, X., Ruan, R., Yuan, X., & Yi, Z. (2018). Effects of nitrogen fertilizer and planting density on the leaf photosynthetic characteristics, agronomic traits and grain yield in common buckwheat (*Fagopyrum esculentum* M.). *Field Crops Research*, 219, 160 - 168.

Fei, L., Hongdong Y, Zhenguo, W., Tao, L, Guangyu, F, Yuemei, R., Yu, Z., Guangbing, R., Wenjuan, Z., Yanxi, J., Hongkui, L., Yan, H., Guoliang, S., Mo, L., Zhilan, D., Defeng, S., Fanhua, M,, Fangchao, G., Fenxia, H., Peng, Y., Jihong, L. & Guiying, L. (2025). Interpretation of genotype-environment-nitrogen management interactions in the early maturing sorghum (*Sorghum bicolor* (L.) Moench) region of China, *Journal of Agriculture and Food Research*,19.

Food and Agriculture Organization of the United Nations (FAOSTAT) (2024). Sorghum production quantity by country, 2022. Crops Statistical database. *Food and Agriculture Organization of the United Nations*.

Fornari, E., Gaviraghi, L., Basso, C., Pinheiro, M., Vian, A., & Santi, A. (2020). Relationship between photosynthetic pigments and corn production under nitrogen sources. *Pesquisa Agropecuária Tropical*.

Ferguson, J. N., Schmuker, P., Dmitrieva, A., Quach, T., Zhang, T., Ge, Z., Nersesian, N., Sato, S. J., Clemente, T. E., & Leakey, A. D. B. (2024). Reducing stomatal density by expression of a synthetic epidermal patterning factor increases leaf intrinsic water-use efficiency and reduces plant water use in a C_4 crop. *Journal of Experimental Botany*, 75(21), 6823 – 6836.

Hashimoto, H., Uragami, C., & Cogdell, R. J. (2016). Carotenoids and photosynthesis. Sub-Cellular Biochemistry. 79, 111 – 139.

Hariprasanna, K., & Rakshit, S. (2016). Economic importance of sorghum. In S. Rakshit & Y. H. Wang (Eds.), The sorghum genome. 1 – 25.

Hiscox, J. D., & Israelstam, G. F. (1979) A Method for Extraction of Chlorophyll from Leaf Tissue without Maceration. *Canadian Journal of Botany*, 57, 1332-1334.

Holman, J. D., Schlegel, A. J., Thompson, C. R., & Stone, L. R. (2024). Nitrogen fertiliser source, rate, placement and application timing effect on sorghum grain and forage and corn grain yields. *Agrosystems, Geosciences & Environment*, 7(1), 20469.

Ismail, R., Shamshuddin, J., Che Ishak, C., & Anuar, A. R. (2011). Fertility and suitability of the Spodosols formed on sandy beach ridges interspersed with swales in the Kelantan–Terengganu Plains of Malaysia for kenaf production. *Malaysian Journal of Soil Science*, 15, 1 – 24.

Kazungu, F. K., Muindi, E. M., & Mulinge, J. M. (2023). Overview of Sorghum (*Sorghum bicolor*. L), its economic importance, ecological requirements and production constraints in Kenya. *International Journal of Plant & Soil Science*. 62 – 71.

Lake, J. A., & Woodward, F. I. (2008), Response of stomatal numbers to CO_2 and humidity: control by transpiration rate and abscisic acid. *New Phytologist*. 179, 397 - 404.

Li, Y. Y., Ming, B., Fan, P. P., Liu, Y., Wang, K. R., Hou, P., & Xie, R. Z. (2022). Effects of nitrogen application rates on the spatio-temporal variation of leaf SPAD readings on the maize canopy. *The Journal of Agricultural Science*, 160(1–2), 32 – 44.

Makino, Y., & Ueno, O. (2018). Structural and physiological responses of the C_4 grass *Sorghum bicolor* to nitrogen limitation. *Plant Production Science*. 21(1), 39 – 50.

Martiwi, I. N. A., Nugroho, L. H., Daryono, B. S., & Susandarini, R. (2020). Morphological variability and taxonomic relationship of *Sorghum bicolor* (L.) Moench accessions based on qualitative characters. *Annual Research & Review in Biology*. 40 – 52.

Markwell, J., & Namuth, D. (2003). Plant pigments and photosynthesis. *Journal of National Resource Life Science Education*. 32, 137 – 137.

Moeinirad, A., Zeinali, A., Galeshi, S., Afshin, S., & Eganepour, F. (2021). Investigation of fluorescence chlorophyll sensitivity, chlorophyll index, rate of Chlorophyll (a, b), nitrogen concentration and nitrogen nutrition index under under nitrogen and phosphorus nutrition in wheat. *Journal Crop Production*. 14(1), 1 – 18.

Mustaffer, N., Jalani, S. I., Devarajan, T., & Hamid, Ahmad. (2024). Evaluation of new sorghum varieties for grain production cultivated in mineral soil. *World Journal of Advanced Research and Reviews*. 23. 1606 - 1611.

Nguyen, T. P., & Sung, J. (2025). Light spectral-ranged specific metabolisms of plant pigments. *Metabolites*, 15(1), 1.

Ostmeyer, T. J., Bahuguna, R. N., Kirkham, M. B., Bean, S., & Jagadish, S. V. K. (2022) Enhancing Sorghum Yield Through Efficient Use of Nitrogen; Challenges and Opportunities. *Frontier Plant Science*, 13:845443.

Paul, M. J., & Driscoll, S. P. (1997). Sugar repression of photosynthesis: the role of carbohydrates in signalling nitrogen deficiency through source: sink imbalance. *Plant, Cell & Environment*, 20(1), 110 – 116.

Paul, M. J., & Foyer, C. H. (2001). Sink regulation of photosynthesis. *Journal of Experimental Botany*, 52(360), 1383 – 1400.

Peng, J., Feng, Y., Wang, X., Li, J., Xu, G., Phonenasay, S., Luo, Q., Han, Z., & Lu, W. (2021). Effects of nitrogen application rate on the photosynthetic pigment, leaf fluorescence characteristics, and yield of indica hybrid rice and their interrelations. *Scientific Reports*. 11(1).

Piccinni, G., Ko, J., Marek, T., & Howell, T. (2009). Determination of growth-stage-specific crop coefficients (Kc) of maize and sorghum. *Agricultural Water Management*, 96(12), 1698 – 1704.

Qi, Z., Dong, Y., He, M., Wang, M., Li, Y., & Dai, X. (2021). Coated, stabilised enhanced-efficiency nitrogen fertilisers: Preparation and effects on maize growth and nitrogen utilisation. *Frontiers in Plant Science*, 12, Article 792262.

Razali, R., & Salmizi, A. (2023). Improving the BRIS Soil management and practices for growing roselle (*Hibiscus sabradiffa* L.). *Journal of Tropical Resources and Sustainable Science* (JTRSS).

Sanjana, R. P. (2017). Sorghum, *Sorghum bicolor* (L.) Moench. In J.V. Patil (Ed.), Millets and Sorghum: Biology and Genetic Improvement. *ICAR-Indian Institute of Millets Research, Hyderabad*, India. 1 – 48.

Sarshad, A., Talei, D., Torabi, M., Rafiei, F., & Nejatkhah, P. (2021). Morphological and biochemical responses of *Sorghum bicolor* (L.) Moench under drought stress. *SN Applied Sciences*, 3(1).

Shah, A., Yang, G., Tanveer, M. & Iqbal, J. (2017). Leaf gas exchange, source–sink relationship, and growth response of cotton to the interactive effects of nitrogen rate and planting density. *Acta Physiologiae Plantarum*, 39.

Shi, D., Zhuang, K., Chen, Y., Xu, F., Hu, Z. & Shen, Z. (2020). Effects of excess ammoniacal nitrogen ($NH_{4+-}N$) on pigments, photosynthetic rates, chloroplast ultrastructure, proteomics, formation of reactive oxygen species and enzymatic activity in submerged plant *Hydrilla verticillata* (L.f.) Royle. *Aquatic Toxicology*, 105585.

Soleymani, A., Shahrajabian, M. H. & Naranjani, L. (2011). The effect of plant density and nitrogen fertilisation on yield, yield components and grain protein of grain sorghum. *Journal of Food, Agriculture and Environment*. 9. 244 - 246.

Sun, J., Li, W., Li, C., Chang, W., Zhang, S., Zeng, Y., Zeng, C. & Peng, M. (2020). Effect of different rates of nitrogen fertilisation on crop yield, soil properties and leaf physiological attributes in banana under subtropical regions of China. *Frontiers in Plant Science*, 11.

Thilakarathna, R. C. N., Madhusankha, G. D. M. P. & Navaratne, S. B. (2022). Potential food applications of sorghum (*Sorghum bicolor*) and rapid screening methods of nutritional traits by spectroscopic platforms. *Journal of Food Science*. 87(1), 36 – 51.

Tranavičienė, T., Šikšnianienė, J. B., Urbonavičiūtė, A., Vagusevičienė, I. & Samuolienė, G. (2007). Effects of nitrogen fertilisers on wheat photosynthetic pigment and carbohydrate contents. *Biologija*. 53,80 - 84.

Tranavičienė, T., Urbonavičiūtė, A., Samuolienė, G., Duchovskis, P., Vagusevičienė, I. & Sliesaravičius, A. (2007). The effect of differential nitrogen fertilisation on photosynthetic pigment and carbohydrate contents in the two winter wheat varieties. *Biologija*. 53(4), 80 - 84.

Tenywa, M. M., Nyamwaro, S. O., Kalibwani, R., Mogabo, J., Buruchara, R. & Oluwole, F. (2018). Innovation opportunities in sorghum production in Uganda.

Xue, L. & Yang, L. (2009). Deriving leaf chlorophyll content of green-leafy vegetables from hyperspectral reflectance. *Journal of Photogrammetry and Remote Sensing*, 64(1), 97 – 106.

Yuan, L., Tang, Y., Wu, L. & Peng, Y. (2021). Colorimetric estimation of leaf pigment content and photosynthetic potential in nitrogen-treated rice. *Plant Physiology and Biochemistry*, 164, 59 – 68.

Zhao, D., Reddy, K. R., Kakani, V. G. & Reddy, V. R. (2005). Nitrogen deficiency effects on plant growth, leaf photosynthesis, and hyperspectral reflectance properties of sorghum. *Eur. Journal Agronomy*. 22, 391 – 403.

Zhao, Y., Wang, S., Liu, B. & Guo, W. (2022). Estimating leaf nitrogen content using CIE Lab colour space in maize. *Precision Agriculture*, 23, 1081 – 1097.