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Research Article

Growth and Yield Response of BAU Chia-1 (*Salvia hispanica*) to Nitrogen Fertilizer

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ABSTRACT

Chia (*Salvia hispanica* L.**)** is a newly introduced drought-tolerant crop in Bangladesh and registered as BAU chia-1 in 2019 for countrywide cultivation. Its seeds are considered a superfood because of having a large amount (57-65%) of α-linolenic acid (ω-3 fatty acid). This study aims to determine the amount of optimum nitrogen fertilizer to get a good yield in Bangladesh. A field experiment was conducted from November 2023 to February 2024, to assess the influence of nitrogen in the growth and yield of BAU chia-1. A randomized complete block design was conducted with four nitrogen fertilizer treatments (T_0 = control/ 0 kg N ha^{-1}, T_1= 90 kg N ha^{-1}, T_2 = 120 kg N ha^{-1}, T_3= 150 kg N ha^{-1}) with three replications. The vegetative growth (plant height, fresh weight $plant^{-1}$, dry weight $plant^{-1}$, leaf number $plant^{-1}$) was higher in the case of T_3, but in case of yield contributing attributes (number of inflorescence $plant^{-1}$, main inflorescence length, number of spikes $inflorescence^{-1}$, number of spikelet $spike^{-1}$) and seed yield T_2 showed the highest value. Though, SPAD was also the highest in T_3, leaf area and fluorescence was higher in T_2 than others. Finally, main inflorescence length (39.4 cm), number of inflorescences $plant^{-1}$ (21), spikes main $inflorescence^{-1}$ (20), spikelet $spike^{-1}$ (24) and seed yield (1066 kg ha^{-1}) were higher in T_2 treatment than the others. The optimal nitrogen level for BAU chia-1 cultivation appears to be 120 kg N ha^{-1}. However, further multi-location trials are required to validate these findings before making any recommendations.

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Introduction

Chia (*Salvia hispanica* L.) is an annual herbaceous plant belonging to the Lamiaceae family (Hossain et *al.* 2016). This crop is native to the mountain areas of Mexico and Guatemala (Ixtaina *et al.,* 2008; Sosa-Baldivia *et al.,* 2018) and was an important staple crop during pre-Columbian times in Mexico (Cahill, 2003). It was first introduced in Bangladesh, in 2010 and registered as BAU chia-1 by the Ministry of Agriculture in 2019 (Shorna, 2024). This macro-thermal short-day species produces purple or white flowers and brown or white colour seeds with oppositely arranged leaves. Its seed is oval-shaped and small in size, a couple of millimetres in length (Muñoz *et al.,* 2013). However, chia is gaining popularity due to its high nutritional value, especially the presence of ω-3 fatty acid (~67%) and dietary fibre. According to USDA Food Data Central, 100g of chia seed contains 17.8g of Polyunsaturated Fatty Acid (ALA; 18:3). Additionally, it contains protein (16.5g $100g^{-1}$), lipid (30.7g $100g^{-1}$), carbohydrate (42.1 g $100g^{-1}$), dietary fibre (42.1g $100g^{-1}$), minerals (Ca, Fe, Mg, P, K etc.) and so on. Moreover, some researchers found that phenolic and antioxidant substances are high in chia seeds (da Silva Marineli *et al.,* 2014). The potential health benefit of chia was reported by a group of pharmaceuticals and nutraceuticals researchers (Ulbricht *et al.,* 2009; Ali *et al.,* 2012; Katunzi-Kilewela *et al.,* 2021). This is the only reason the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) announced chia seeds as novel foods (NFs) under Regulation (EU) 2015/2283 (Dinçoğlu *et al.,* 2019; Suri *et al.,* 2015).

The area under cultivation of BAU chia-1 is expected to rise in the coming days as it requires less water and is a drought-resistant crop that comes up very well under adverse climatic conditions (Harisha *et al.,* 2023). However, being a new crop, the optimization of different agronomic management practices is very scarce under Bangladesh conditions, which is necessary

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for harvesting maximum yield. Very few works addressed the effect of sowing date (Karim *et al.* 2015), sowing method (Biswas *et al.* 2020) and sowing density (Sumi *et al.* 2023) or sowing density in combination with fertilization strategies (Rahman *et al.* 2023) for good harvest of BAU chia seed in the climatic condition of Bangladesh. In addition to above mentioned agronomic practices, nitrogen fertilization plays a critical role concerning seed yield and seed quality traits. An assessment of optimized nitrogen fertilization is necessary to evaluate the direct effects on plant growth, maturity, yield contributing characters and seed yield as well. Therefore, the present study aimed to evaluate the response of BAU chia-1 to nitrogen fertilization under subtropical conditions in Bangladesh.

Materials and Methods

The study investigated the growth, yield, and physiological characteristics of BAU chia-1 under different nitrogen treatments at the Crop Botany Research Field of Bangladesh Agricultural University, Mymensingh. The seeds were authenticated and the experiment was conducted from November 2023 to February 2024. The field was prepared by tilling and levelling, followed by the application of fertilizers: 20 kg N ha^{-1} as urea, 40 kg P ha^{-1} as TSP, and 20 kg K ha^{-1} as MoP. The remaining nitrogen fertilizer was top dressed at 30 DAS according to the experimental design.

The experiment followed a Randomized Complete Block Design with four nitrogen levels (T_0 = control / 0 kg N ha^{-1}, T_1 = 90 kg N ha^{-1}, T_2= 120 kg N ha^{-1}, T_3= 150 kg N ha^{-1}) and three replications. As a basal dose, 20 kg of nitrogen is initially applied to the field. After 30 days, the remaining amount of nitrogen, as specified by the experimental design, is spread over the field. Each plot measured 20 m^2 (5m × 4m). The plots were arranged in three blocks based on similar soil characteristics, and treatments were randomly assigned within each block. Seeds were sown at the rate of 2.5 kg ha^{-1} following broadcasting method. The crop was grown without irrigation due to sufficient soil moisture and managed manually for weeds at 25 and 40 DAS. No pesticides were applied because pest infestations were minimal.

Parameters recorded during the growing period included plant height, root length, fresh and dry weight $plant^{-1}$, leaf number, leaf area, and SPAD value (a measure of chlorophyll content). Measurements of plant height and root length were taken using a centimeter ruler, while fresh and dry weights were recorded using a digital scale. Plants were dried at 70°C for 72 hours to determine dry weight. The leaf area was measured using an LI-3100 Area Meter, and chlorophyll content was assessed with a SPAD 502 plus chlorophyll meter. The potential quantum yield of PSII in dark-adapted leaves (Fv/Fm) was measured at 10-day intervals from 30 DAS using a Hansatech Pocket PEA Chlorophyll Fluorimeter.

At harvest, yield contributing parameters such as the number of inflorescences $plant^{-1}$, main inflorescence length, number of spikes $inflorescence^{-1}$, number of spikelets $spike^{-1}$, and seed yield were recorded from 10 plants of each replication. The total number of leaves, inflorescences, and spikes were counted from randomly selected plants in each plot. After harvesting, seeds were cleaned, and the seed yield was measured.

Table 1. Effect of nitrogen fertilizer level on plant height of chia at different days after sowing (DAS)

N level (Kg ha^{-1})	Plant height (cm)									
	Days after Sowing (DAS)									
	10	20	30	40	50	60	70	80	90	100
0 (Control)	3.7±0.21^{b}	16.1±0.4^{ab}	30.8±3.2^{a}	47.5±0.7^{c}	63.3±1.8^{d}	85.8±2.8^{b}	102.7±1.7^{a}	107.7±5.6^{b}	110.3±1.5^{c}	128.9±3.1^{b}
90	5.3±0.08^{a}	16.9±0.2^{ab}	32.8±1.1^{a}	55.7±0.6^{b}	76.2±0.9^{c}	111.5±2.4^{a}	113±2.7^{a}	116.1±0.6^{ab}	134.4±2.2^{b}	139.5±1.04^{b}
120	4.4±0.12^{b}	17.7±0.08^{a}	29.1±1.4^{a}	65.7±1.9^{a}	84.8±2.2^{b}	105.4±7.5^{a}	113.7±1^{a}	115.5±1.7^{ab}	132.3±1.2^{b}	151.7±1.7^{a}
150	5.5±0.15^{a}	14.4±1.05^{b}	34.1±4.9^{a}	63.4±1.6^{a}	89.5±1.3^{a}	108.3±2.8^{a}	113±4.3^{a}	124.6±2.02^{a}	147.7±1.5^{a}	160.6±1.2^{a}
CV (%)	17.92	8.70	6.89	14.19	14.68	11.27	4.75	5.96	11.81	9.54

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Statistical analysis

The collected data were analyzed statistically following the analysis of variance (ANOVA) technique and the mean differences were adjudged by Duncan's Multiple Range Test (DMRT) using the statistical computer package program Minitab.

Results and Discussion

Plant height

In this study, chia plant height was measured at 10-day intervals that were treated with nitrogen fertilizer at 30 DAS. Initially, the plant's height was uniform but by 40 DAS, differences observed due to nitrogen application (Table 1). However, at 40 DAS, the observed plant height was 47.5 cm, 55.7 cm, 65.7 cm, and 63.4 cm in control, T_1, T_2, and T_3 respectively. So the timing of nitrogen application is essential for proper vegetative growth. Notably, T_1 showed the tallest plants at 60 DAS (111.5 cm). By 70 DAS, no significant differences were observed in plant height. From 80 DAS to 100 DAS, T_3 had the tallest plant. This observation suggests that higher nitrogen levels boost growth by increasing

proteins and amino acids, which promote cell elongation and multiplication. It is noteworthy that, the chia plant was uprooted for data collection, so the measurement was not obtained from the same individual plants throughout the study. Thus, there may be some variability in this data. This finding aligns with the research of Njoka *et al.,* (2022). According to their research, the application of the highest nitrogen fertilizer increased vegetative growth.

Root length

Root length exhibited an opposite pattern of plant height, showing decreases in treated groups compared to control (Table 2). Initially, there were no significant differences in the root length of chia plants up to the application of nitrogen fertilizer. However, at 40 DAS, the root length of control plots (7.4 cm) surpassed the treated plots (T_1 = 6 cm, T_2= 4.9 cm, T_3 = 6.2 cm). This result may indicate some factors that influence the root development, such as nutrient availability, and physiological responses to external stimuli. The highest nitrogen application (T_3) surprisingly resulted in the shortest (8.9 cm) root length of the chia plant in the final harvest. This may be ascribed the variations in soil nutrient availability (López-Bucio *et al.,* 2023). Furthermore, data collection was not conducted on the same individual plant, similar to plant height.

Table 2. Effect of nitrogen fertilizer level on root length of chia at different days after sowing (DAS)

N level (Kg ha^{-1})	Root length (cm)									
	Days after Sowing (DAS)									
	10	20	30	40	50	60	70	80	90	100
0 (Control)	2.3±0.23^{a}	3.4±0.06^{a}	5.5±0.77^{a}	7.4±0.17^{a}	9.6±0.18^{a}	9.6±0.32^{a}	14.9±0.46^{a}	13.8±0.17^{a}	13.8±0.4^{a}	12.2±0.17^{a}
90	2.4±0.21^{a}	3.6±0.09^{a}	5.2±0.29^{a}	6.0±0.21^{ab}	8.4±0.15^{b}	8.7±0.14^{b}	10±0.40^{b}	9.0±0.12^{b}	8.6±0.58^{b}	8.1±0.64^{b}
120	2.7±0.12^{a}	3.4±0.17^{a}	4.3±0.26^{a}	4.9±0.46^{b}	8.7±0.17^{b}	9.4±0.21^{a}	12.4±0.64^{ab}	10.3±0.35^{b}	11.0±0.55^{b}	10.3±0.94^{ab}
150	2.9±0.03^{a}	3.4±0.54^{a}	5.9±0.25^{a}	6.2±0.14^{ab}	7.8±0.20^{b}	9.3±0.13^{a}	11.9±0.45^{b}	9.2±0.60^{b}	9.1±0.23^{b}	8.9±0.48^{ab}
CV (%)	10.72	3.74	12.99	16.24	9.00	3.99	16.50	20.95	22.27	18.37

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Fresh weight plant^{-1}

The fresh weight plant^{-1}, represented in Figure 1, showed a consistent increase over time in all treatments, though the control plants exhibited lower fresh weights compared to other treatments. The lower value in control plants describes the necessity of nitrogen fertilizer in biomass accumulation. T_3 recorded the highest fresh weight from the beginning to harvesting and T_2 showed the second highest fresh weight. However, there is no significant difference in T_2 and T_3. El-Desouky *et al.,* (2022) put nitrogen as an amino acid and found that the highest fresh weight at the harvesting time was obtained from the higher amino acid application which aligns with this study.

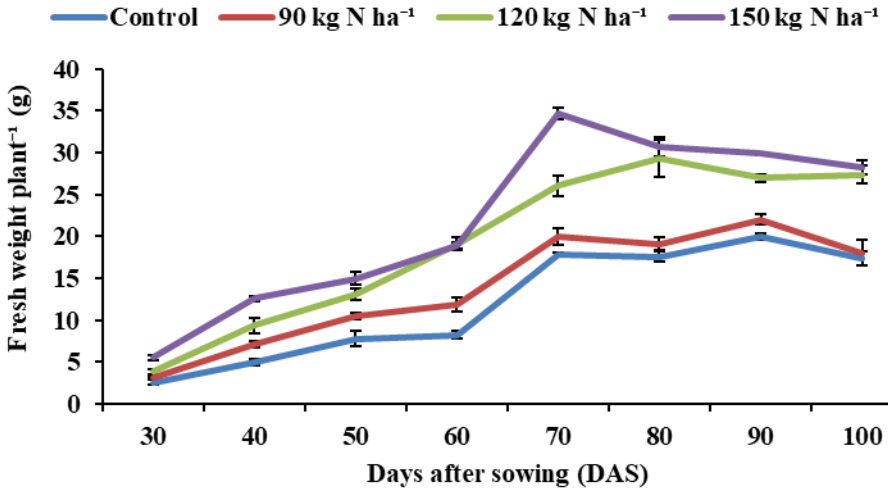


Figure 1. Fresh weight of chia at different growth stages as affected by nitrogen fertilizer level. The vertical bars represent the standard error.

Dry weight plant^{-1}

Plants treated with nitrogen fertilizer always displayed greater dry matter production than the control (Figure 2). At 60 DAS, T_2 showed the highest (3.7 g) dry matter accumulation. Subsequently, at 70 DAS, both T_2 and T_3 demonstrated nearly equivalent (T_2 = 6.7 g, T_3= 6.8 g) dry matter production. However, from 80 DAS up to harvesting, T_3 continuously displayed the highest values

of dry matter accumulation. This indicates that nitrogen fertilizer application is essential for dry matter accumulation. Again, nitrogen fertilizer is a fundamental building block for essential molecules involved in growth and development, which is essential for photosynthesis, respiration and protein synthesis. Moreover, it mitigates nutrient deficiencies, enhances stress tolerance, and improves overall plant vigour, finally contributing to increased dry matter accumulation. Souza and Chavez (2017) found 8.06 g plant^{-1} dry matter after applying 125 kg N ha^{-1} due to the acceleration of cell division and expansion, whereas in this study, the highest dry matter during harvesting was 8.25 g plant^{-1} in T_3. El-Desouky *et al.,*(2022) also mentioned high dry matter accumulation in higher amino acid application. A similar result was recorded by Njoka *et al.,* (2022) that the highest nitrogen application is responsible for higher dry matter accumulation.

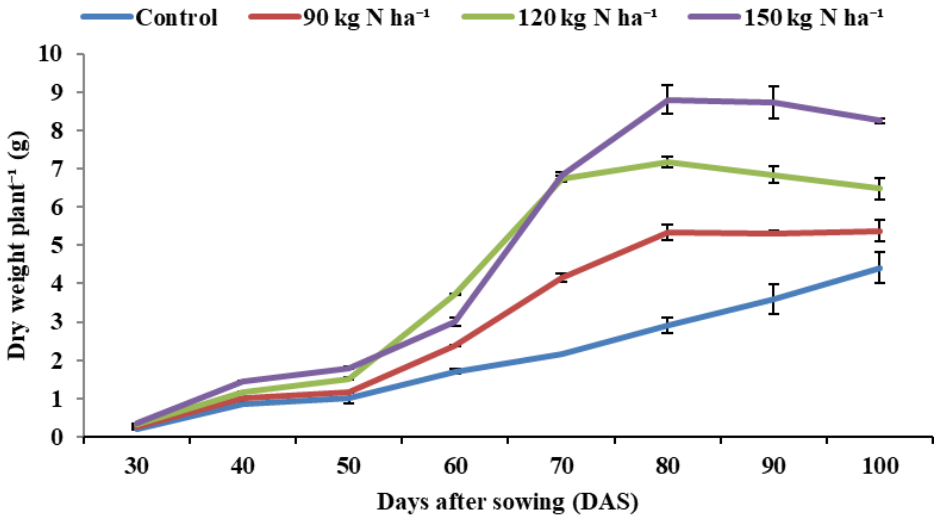


Figure 2. Dry weight of chia at different growth stages as affected by nitrogen fertilizer level. The vertical bars represent the standard error.

Number of leaves plant^{-1}

The ontogeny of the leaf number increment of the chia plant is represented in Table 3. There was less difference in leaf number before nitrogen fertilizer application. After nitrogen fertilizer application, the control plants exhibited inferior leaf numbers. Leaf number was the highest at T_3 all over the growing period. Therefore, a low dose of nitrogen fertilizer may be responsible for lower leaf numbers. At the final harvest, the leaf number of the chia plant in control, T_1, T_2, and T_3 was 36, 41, 47.3, and 56.7, respectively. Adequate nitrogen availability promotes the production of new leaves, so the leaf number increases in higher nitrogen fertilizer application. Njoka *et al.,* (2022) also mentioned higher leaf numbers in higher nitrogen application.

Table 3. Effect of nitrogen fertilizer level on number of leaves plant^{-1} of chia at different days after sowing (DAS)

N level (Kg ha^{-1})	Leaf number Plant^{-1}									
	Days after Sowing (DAS)									
	10	20	30	40	50	60	70	80	90	100
0 (Control)	5.7±0.3^{a}	9±0.6^{a}	12.3±0.3^{b}	17±0.6^{c}	18.3±0.3^{d}	20±0.6^{b}	22.3±1.3^{b}	29.3±2.7^{b}	31.3±3.7^{b}	36±1.2^{b}
90	7±0.6^{a}	8.3±0.7^{a}	15.7±0.9^{a}	20±0.6^{b}	22±0.6^{c}	23±1^{b}	24±0.6^{b}	33.3±2.9^{ab}	35.3±1.7^{ab}	41±3^{b}
120	7.3±1.2^{a}	9±0.6^{a}	15±0.6^{ab}	23±0.6^{a}	26.3±0.3^{b}	31.7±0.9^{a}	34.7±0.7^{a}	38.3±1.8^{ab}	44.7±2.4^{a}	47.3±2.7^{ab}
150	7.3±0.3^{a}	9±0.6^{a}	17±1.5^{a}	20±0.6^{b}	29.7±0.9^{a}	32±1.5^{a}	37±2.5^{a}	42±4.2^{a}	46.7±4.4^{a}	56.7±3.3^{a}
CV (%)	11.61	3.77	13.08	12.25	20.57	22.85	25.11	15.55	18.61	19.69

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SPAD value

Figure 3 demonstrated that plants treated with nitrogen fertilizer had higher SPAD values, indicating increased chlorophyll content compared to the control. Therefore, a consistent pattern of higher SPAD values was observed in T_3, indicating that the higher nitrogen dose enhances optimal chlorophyll synthesis. However, there were inconclusive results in T_1 and T_2. The main factor influencing this variation may be due to N_2 which is the key component of chlorophyll ($C_{55}H_{72}N_4Mg$). Relative chlorophyll content will decrease due to lower nitrogen as it is essential for chlorophyll synthesis (Bidwell, 1974; Lambers *et al.,* 2011). A similar result was also recorded in Esmail *et al.,* (2022), when SPAD value increased in higher nutrient supply.

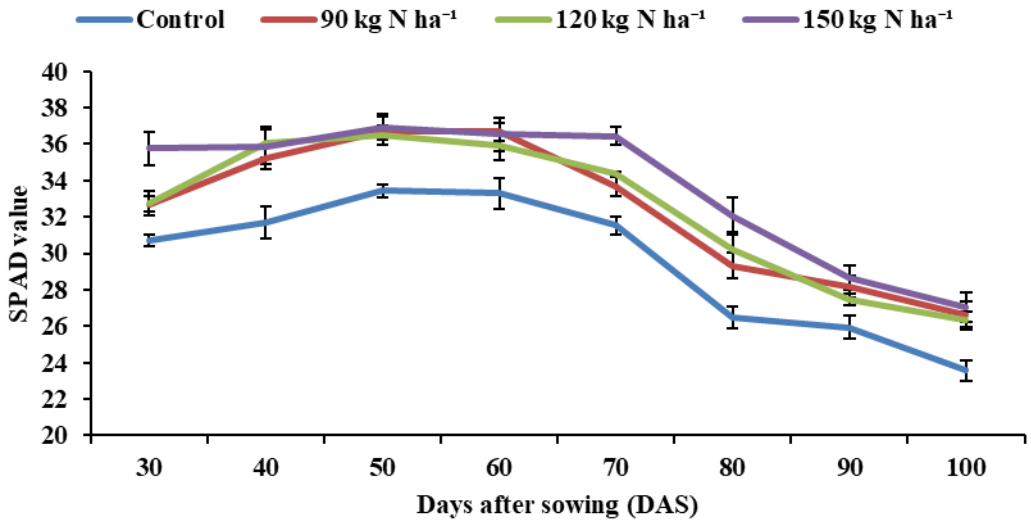


Figure 3. SPAD value of chia at different growth stages as affected by nitrogen fertilizer level. The vertical bars represent the standard error.

Leaf area plant^{-1}

The leaf area of chia was increased gradually till the reproductive stage and declined subsequently (Table 4). This shows the natural pattern of leaf growth and senescence. At 60 DAS, all treatments showed the maximum leaf area, except T_1. Interestingly, at 70 DAS, T_1 showed the highest leaf area, which suppresses T_3. Among all the treatments, T_2 resulted in the highest leaf area till harvesting. This indicates that optimum nitrogen (T_2) is essential for leaf growth and canopy development. A unique result was found at 90 DAS when T_2 and T_3 showed approximately same values. This result reveals that excessive nitrogen isn't always better. In this case, T_2 may be more beneficial for leaf development compared to T_3. Though Mack *et al.,* (2017) revealed that higher nitrogen application (40 kg N ha^{-1}) is responsible for higher leaf area in chia.

Table 4. Effect of nitrogen fertilizer level on leaf area of chia at different days after sowing (DAS)

N level (Kg ha^{-1})	Leaf area (cm^2)							
	Days after Sowing (DAS)							
	30	40	50	60	70	80	90	100
0 (Control)	27.4± 1.8^{c}	36.5± 2.04^{c}	53.5± 2.4^{b}	57± 3.4^{b}	53.5± 2.5^{b}	49.5± 4.6^{b}	42.1± 1.2^{c}	37.9± 0.65^{c}
90	37.8± 3.04^{bc}	42.5± 2.42^{bc}	56.3± 0.53^{b}	66.2± 2.8^{b}	79.8± 3.9^{a}	67.2± 1.05^{a}	50.9± 1.05^{b}	47.2± 0.5^{bc}
120	55.1± 2.7^{a}	70.4± 3.41^{a}	68.9± 1.9^{a}	98.3± 1.9^{a}	79.6± 1.7^{a}	77.9± 2.9^{a}	64.5± 1.04^{a}	63.3± 2.02^{a}
150	49.8± 3.19^{ab}	52.2± 3.49^{b}	60.2± 2.77^{ab}	84.3± 4.2^{a}	68.4± 3.6^{ab}	65.7± 1.8^{a}	63.7± 1.8^{a}	56.8± 3.3^{ab}
CV (%)	29.15	29.30	11.21	24.16	17.64	18.05	19.45	21.73

In a column figures with same letter do not differ significantly.

Fluorescence

This study investigates the impact of varying nitrogen application rates on chlorophyll fluorescence in chia plants (Table 5). Mean Fv/Fm values, indicating photosynthetic efficiency, ranged from 0.66 to 0.79 across nitrogen treatments depicted in Table 5. The control plant had the lowest fluorescence, while T_2 consistently showed the highest levels, indicating optimal PS-II efficiency with higher photosynthetic performance. Also, the leaf area of T_2 was higher than other treatments. Conversely, the lowest fluorescence level was recorded in T_3 among all the treatments, possibly due to physiological stress from excessive nitrogen. Notably, the plants of T_1 showed higher fluorescence levels compared to T_3. At 60 DAS, T_2 demonstrated the highest Fv/Fm, indicating that the photosystems were operating at 81% efficiency during the entire growth period. In contrast, T_1 achieved 78.3% efficiency at 80 DAS, while T_3 maintained approximately 76% efficiency at 50 DAS, 60 DAS, and 70 DAS throughout the growth period. DaMatta *et al.,* (2002) showed that nitrogen deficiency may affect PSII photochemistry because it decreases the quantum yield of PSII electron transport and Fv/Fm. Again, Shelly *et al.,* (2007) demonstrate that NH_4^+ additions produce more complex effects on fluorescence than NO_3^- additions.

Table 5. Effect of nitrogen fertilizer levels on fluorescence of chia at different days after sowing (DAS)

N level (Kg ha^{-1})	Fluorescence							
	Days after Sowing (DAS)							
	30	40	50	60	70	80	90	100
0 (Control)	0.69± 0.01^{a}	0.69± 0.003^{b}	0.73± 0.005^{b}	0.74± 0.008^{b}	0.75± 0.006^{a}	0.74±0.03^{a}	0.72± 0.003^{b}	0.67± 0.02^{a}
T_1	0.70± 0.01^{a}	0.74± 0.02^{ab}	0.76± 0.006^{ab}	0.77± 0.01^{ab}	0.77± 0.03^{a}	0.78± 0.02^{a}	0.76± 0.02^{ab}	0.72± 0.01^{a}
T_2	0.73± 0.02^{a}	0.77± 0.02^{a}	0.79± 0.008^{a}	0.81± 0.005^{a}	0.79± 0.02^{a}	0.79± 0.01^{a}	0.78± 0.007^{a}	0.73± 0.02^{a}
T_3	0.72± 0.01^{a}	0.75± 0.01^{ab}	0.76± 0.006^{ab}	0.76± 0.02^{ab}	0.77± 0.03^{a}	0.75± 0.03^{a}	0.74± 0.02^{ab}	0.71± 0.01^{a}
CV (%)	2.49	4.40	3.06	3.98	2.00	3.65	3.38	3.97

In a column figures with same letter do not differ significantly.

Main inflorescence length

This study found significant variations in the main inflorescence length among different nitrogen fertilizer applications (Figure 4). Plants in the control group exhibited relatively shorter main inflorescence, with an average of 20.33 cm. In contrast, plants of T_2 displayed the longest main inflorescence, with an average of 39.4 cm, which indicates the necessity of nitrogen fertilizer in inflorescence elongation. Interestingly, T_1 showed a slightly lower (27.9 cm) average main inflorescence length compared to T_2. Further, the main inflorescence length was intermediate in T_3 treatment, averaging 33.2 cm. This indicates that excessive nitrogen application may not be necessary for the increase of main inflorescence length and lower nitrogen availability may stimulate the length. The moderate nitrogen fertilizer application can influence inflorescence elongation. The length was recorded 17.8 cm where no additional fertilizer was applied (Biswas *et al.,* 2020), whereas Rahman *et al.,* (2023) recorded the inflorescence length above 30 cm where different doses of fertilizers was applied.

Number of inflorescences plant^{-1}

Figure 4 shows a significant impact of different nitrogen levels on inflorescence development. Chia plants with no nitrogen application exhibited an average of 9 inflorescences per plant. However, as nitrogen dosage increased, there was variation in inflorescence production. Specifically, plants of T_1 showed an average of 6.66 inflorescences plant^{-1}. Further, T_3 resulted in an average of 11.33 inflorescences plant^{-1}. The number of inflorescences was 21 in T_2, and it was the highest among the treatments. This finding indicates that optimum nitrogen fertilizer is essential to maximize reproductive development, and excessive nitrogen may not be used by the plants or leaching out to the soil (Jensen *et al.,* 2005).

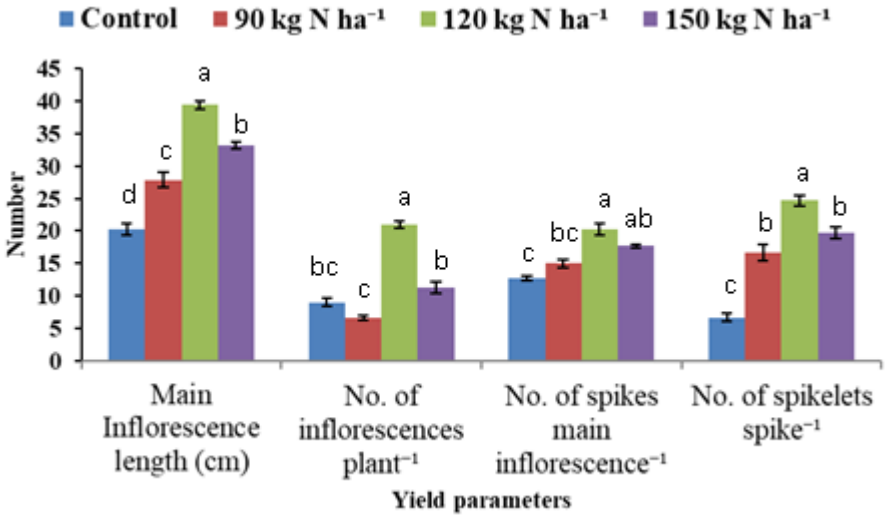


Figure 4. Main inflorescence length, No. of inflorescence number plant^{-1}, No. of spike main inflorescence^{-1}, and No. of spikelet spike^{-1} of chia at different growth stages as affected by nitrogen fertilizer level. The vertical bars represent the standard error (n=3).

Number of spikes main inflorescence^{-1}

This study showed variations in spike production in the chia plant over different nitrogen fertilizer applications delineated in Figure 4. Control plants showed the lowest (average 12.66) spikes main inflorescence^{-1}. T_1 resulted in an increased average of 15 spikes main inflorescence^{-1}. Furthermore, plants of T_2 exhibited a substantial rise in spike production, averaging 20.33

spikes main inflorescence^{-1}. Similarly, T_3 showed an average of 17.66 spikes main inflorescence^{-1}. This finding indicates the importance of optimum nitrogen fertilizer application in spike formation (Marschner, 2012).

Number of spikelets spike^{-1}

There is a significant difference in different levels of nitrogen fertilizer application on spikelet production spike-1 (Figure 4). Control plants showed an average of 6.66 spikelets spike-1. With an average of 16.66 spikelets spike-1 was seen in T1. Additionally, plants of T2 showed a significant increase in the number of spikelets production – an average of 24.66 spikelets spike-1. Also, 19.66 spikelets on average were produced in T3. Biswas et al., (2020) recorded about 16 spikelet spike-1 in chia plants which justify this result.

Seed yield

Doses of nitrogen fertilizer significantly impacted the seed yield of BAU chia-1 displayed in Figure 5. The lowest seed yield, 530kg ha^{-1} was recorded in the control. As the nitrogen doses increased, the yield of T_1 rose to 750 kg ha^{-1}. Further, at T_2, the yield significantly increased to 1066 kg ha^{-1}. However, the yield of T_3 decreased to 750 kg ha^{-1}. It meant that there was no additional benefit observed in the case of a higher dose of nitrogen fertilizer application. Our results are in agreement with the findings of Singh *et al.,*(2023), who got 1020 kg ha^{-1} seeds in higher nitrogen application, and 709 kg ha^{-1} seeds in lower nitrogen fertilizer application.

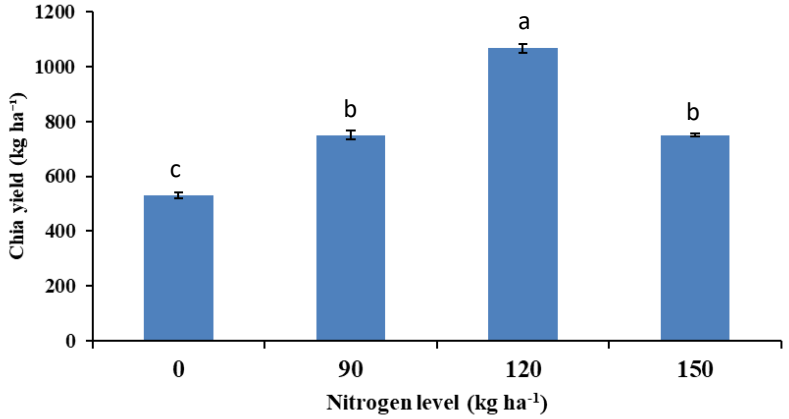


Figure 5. Seed yield of chia at different growth stages as affected by nitrogen fertilizer level. The vertical bars represent the standard error (n=3).

Conclusion

This study confirms the importance of optimum nitrogen fertilizer dose, which influenced different agronomic parameters and yield components in BAU chia-1 grown in Bangladesh. 150 kg N ha^{-1} played a significant role in increasing vegetative growth and biomass accumulation. However, the yield potential was significantly the highest at 120 kg N ha^{-1}. Therefore, 120 kg N ha^{-1} seems to be the best nitrogen level for BAU chia-1 cultivation. Further multilocation trials are needed to confirm this finding before making any recommendations.

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Research Article

Effect of Seed Rates on Growth and Yield of BAU Chia-1 (*Salvia hispanica* L.)

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ABSTRACT

BAU Chia-1, registered in 2019 in Bangladesh for countrywide cultivation, is considered a superfood due to its high amount of ω-3 fatty acids. A field experiment was conducted from November 2024 to February 2025 to evaluate the effect of different seed rates on the growth, reproductive traits, and yield performance. Four seed rate treatments (T_1 = 200 g ha^{-1}, T_2 = 250 g ha^{-1}, T_3 = 300 g ha^{-1}, and T_4 = 350 g ha^{-1}) were assessed with three replications and a Randomized Complete Block Design (RCBD). T_3 produced the tallest plant (145.067 cm), the highest fresh weight (28.67 g), and dry weight (8.1 g). The efficiency of photosynthetically active radiation and the number of leaves per plant were the highest in the T_4 treatment. On the other hand, canopy spread (44.67 cm) and inflorescence number $plant^{-1}$ (8) were the highest in T_1. However, spikelet $spike^{-1}$ was almost similar (7) in T_1 and T_2 seed rates. Despite that, the highest seed yield (1116.67 kg ha^{-1}) was recorded in T_2, followed by T_3 (1083 kg ha^{-1}), T_4(933 kg ha^{-1}), and T_1(883 kg ha^{-1}). In contrast, a lower population may be influenced by a lower seed rate in T_1, whereas reduced growth and yield parameters from higher seed rates (T_4) may be associated with increased intraspecific competition. Taken all together, the seed rate of 250 g ha^{-1}, designated as T_2, showed the most effective crop performance and productivity at the BAU research field in Mymensingh. However, further multi-location trials are required to validate these findings before making any recommendations.

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Introduction

Chia (*Salvia hispanica* L.), a member of the Lamiaceae family, is globally recognized as a superfood for its multiple health benefits and diverse applications. This ancient crop originates from Mexico and Guatemala, contains approximately 30-34% oil, with a high proportion of alpha-linolenic acid (ALA), and essential omega-3 fatty acids that contribute to cardiovascular health (Hossain *et al.,* 2016; Biswas *et al.,* 2023). The seeds are also rich in dietary fiber (34-40%), which aids digestion and helps in weight management (Nadem *et al.,* 2024). Additionally, chia contains essential minerals such as calcium, phosphorus, magnesium, and potassium, making it a highly nutritious food source (Ikumi *et al.,* 2023). Apart from its nutritional significance, chia is well known as a climate-resilient crop. Being drought-tolerant, this short-duration crop can be grown without irrigation or with minimal irrigation using a reduced amount of fertilizer, making it suitable for sustainable and climate-smart agriculture (Hossain, 2023; Harisha et al., 2023; Shorna *et al.,* 2024). Its adaptability to different soil types and minimal input requirements make it an attractive crop to the farmers. This crop needs no pesticides at all (Njoka *et al.,* 2024). Thus, the rising concerns about food security and environmental sustainability, promoting chia cultivation through improved agronomic practices, can contribute to global and national food systems' resilience.

Several field trials have been done in Bangladesh based on agronomic practices of Chia cultivation (Karim *et al.,* 2015; Biswas *et al.,* 2020; Rahman *et al.,* 2023). Recently, it was reported that a plant density of 66 plants m^{-2} of BAU Chia performed well (Sumi *et al.,* 2023). However, very few works are available focused on seed rates, though it is a fundamental agronomic factor that affects plant establishment, intra-crop competition, yield, and farmers' income. Understanding the relationship between seed rate and plant growth is

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essential for developing precise recommendations to enhance productivity while minimizing input costs (McDonald and Copeland, 2012). A lower seed rate results in fewer plants per unit area, reducing competition for water, nutrients, and light. This promotes better vegetative growth, increased branching, and higher seed production per plant. However, lower seed rates can encourage weed growth, increase production cost, and reduce overall yield per hectare. Conversely, a higher seed rate leads to more plants per unit area, intensifying competition for essential resources and potentially limiting individual plant growth and biomass accumulation (Xiao *et al.,* 2025). Optimizing the seed rate is essential for achieving a balanced plant density, enhancing growth efficiency, and maximizing yield. Nowadays, the Market demand for BAU chia-1 is increasing, which leads farmers to cultivate this crop on their lands. However, the lack of standardized recommendations for the seed rate of BAU chia-1 makes it necessary to identify a suitable seed rate for achieving optimal planting density. Accordingly, the study aims to evaluate the effects of different seed rates on the growth of BAU chia-1, its yield components, and overall productivity. By identifying the most suitable seed rate, this research will contribute to improving BAU Chia 1 productivity, reducing input costs, and enhancing farmers' income. The outcomes will not only benefit local farmers but also contribute to the national goal of sustainable agriculture and food security.

Materials and methods

BAU Chia-1, registered in 2019 by the Ministry of Agriculture, was used in this study. This research was conducted from November 2024 to February 2025 at the Crop Botany Research Field, Bangladesh Agricultural University, Mymensingh, Bangladesh. This region is situated at a latitude of 24°38´ N, a longitude of 90°50´ E, and an elevation of 18 m above mean sea level. It has a semiarid climate. Between November 2024 and February 2025, Mymensingh experienced temperatures ranging from 30°C to 16°C. The Bangladesh Meteorological Department reported monthly relative humidity levels within 77% to 79%. The site belongs to the Sonatola series of the Old Brahmaputra Floodplain AEZ-9. The soil at the experimental site was clay loam. The experimental land was prepared using a power tiller and leveled with a ladder. Fertilizer was applied after the previous crop stubble and weeds were cleared. A balanced fertilizer of 90 kg urea, 40 kg TSP, and 20 kg MoP was applied for nitrogen, phosphorus, and potassium, respectively. Urea was applied in split doses: half at sowing and half at the vegetative stage (45 DAS). As soil moisture was sufficient, irrigation was not given throughout the crop growth period. Weeds were manually eliminated at 25 DAS and 40 DAS. The insect pest infestations were negligible.

The experiment was laid out in a Randomized Complete Block Design (RCBD) with four treatments and three replications. The treatments were T_1 = 200 g ha^{-1}, T_2 = 250 g ha^{-1}, T_3 = 300 g ha^{-1}, and T_4 = 350 g ha^{-1}. Each plot measured 3m × 3m, with a buffer zone of 1m between plots for cultural practices. BAU chia-1 seeds were sown using the broadcasting method, ensuring uniform distribution across the plots. The seeds were lightly covered with soil to facilitate germination. Regular field visits were conducted to assess crop growth and development throughout the growing season. BAU chia-1 was ready for harvesting when the vibrant purple flower stalk began to desiccate and the petals had shed.

Plant height, root length, fresh weight, dry weight, and photosynthetically active radiation efficiency (PAR efficiency) were recorded during the growing period. At 70 Days after sowing (DAS), leaf number, starting branches at the nodal position, and canopy spread were recorded. Canopy spread was measured using a measuring tape from edge to edge of the. branches and expressed as cm. At harvesting time, some yield-related parameters were recorded, such as inflorescence number plant^{-1}, main inflorescence length (inflorescence in main stem), spikes per main inflorescence, spikelets per spike, and seed yield. Photosynthetically active radiation efficiency (PAR) was calculated using the following formula –

PAR efficiency (%) =

$$\frac{(PAR\,at\,top\,canopy - PAR\,at\,ground\,canopy)}{PAR\,at\,top\,canopy} \times 100$$

Data were initially organized using Microsoft Excel 2021 and statistically analyzed using Minitab 21 software. One-way ANOVA of the agronomic traits was performed at the 0.05 significance level.

Result and discussion

Plant height

Plant height was significantly influenced by different seed rates and measured at 30, 40, 50, 60, 70, 80, and 90 DAS, as shown in Figure 1. At 30 DAS, the tallest plant (37.67 cm) was recorded under T_4 treatment, while the shortest (24.67 cm) was observed in T_1 treatment. However, T_4 consistently resulted in the tallest plants up to 50 DAS. From 60 DAS, T_3 produced the tallest plant up to 90 DAS. The plant height under the T_3 treatment reached a maximum of 145.1 cm, followed by the T_4 (141.9 cm), T_2 (135.2 cm), and T_1 (131.27 cm) treatments at 90 DAS. Plant height increased progressively with time across all seed rates, but the rate of increase varied significantly among different seed rates. However, the tendency to a

towering type of plant height with increasing seed rate suggests that there may be competition among plants for light, which has stimulated vertical growth (Stanghellini and Katzin, 2024; Chauvel *et al.,* 2005). Although the plants under T_3 treatments exhibited the most vigorous growth, a slight decrease in plant height was observed in T_4 from 60 DAS to 90 DAS. The inverse relationship between T_4 and plant height may be due to excessive intraspecific competition among the chia plants (Yang *et al.,*2024).

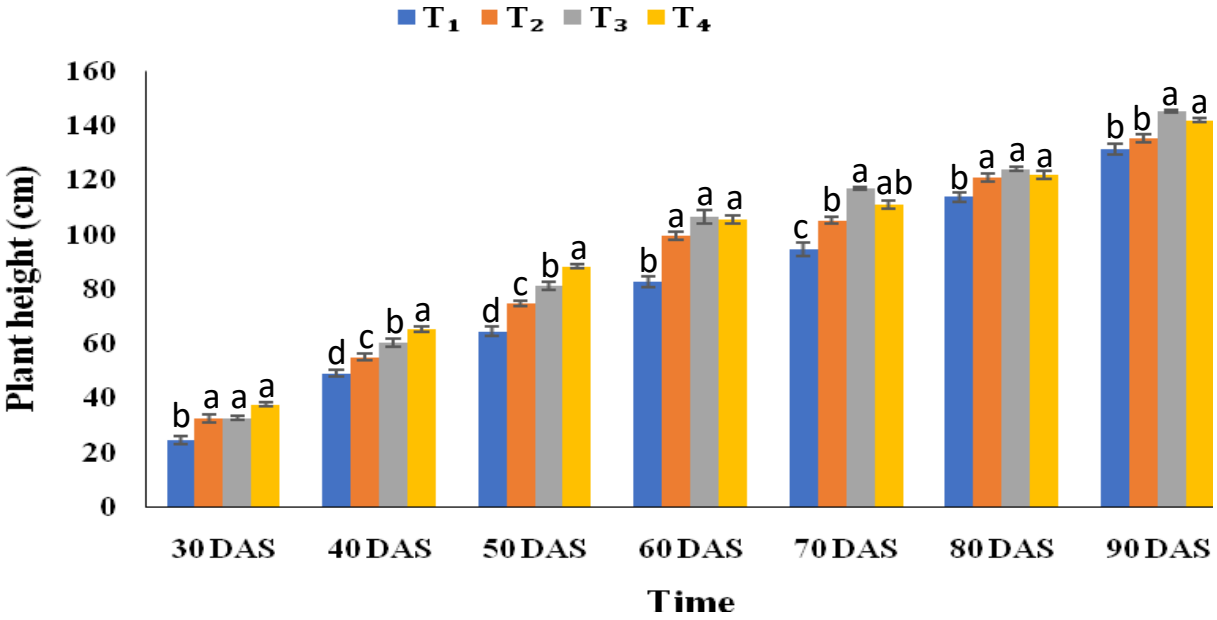


Figure 1: Plant height of BAU chia-1 at different growth stages under different seed rate levels. The vertical bars represent the standard error. Here, T_1 = 200 g ha^{-1}, T_2 = 250 g ha^{-1}, T_3 = 300 g ha^{-1}, and T_4 = 350 g ha^{-1}.

Dry weight plant^{-1}

able 1 shows the effect of four seed rates on different growth stages. At 30 DAS, dry weight ranged from 0.63 g (T_1) to 0.77 g (T_3 and T_4). The dry weight peaked at 80 DAS, with the highest value observed under T_3 (8.9 g), followed by T_4 (8.56 g), T_2 (7.2 g), and T_1 (6 g). A slight decrease in dry weight was noticed at 90 DAS across all treatments, although T_3 still maintained the highest dry weight (8.1 g), while T_1 showed the lowest (5.73 g). The peak observed at 80 DAS denotes the phase of maximum vegetative and early reproductive growth, during which dry matter accumulation typically reaches its maximum (so-called physiologically matured annual crop plant). The slight reduction in dry weight at 90 DAS across all seed rates may be attributed to the onset of senescence, natural decline in tissue moisture, and biomass, similar to fresh weight (Thomas, 2013). Although T_4 showed relatively high dry weight, it was slightly lower than T_3, suggesting that a higher seed rate may cause inefficiencies in resource utilization, as reported in Sumi *et al.,* (2023). Thus, T_3 appears optimal for maximizing dry weight and fresh weight as well. Dalley *et al.,* (2004) also reported that less competition produces higher biomass.

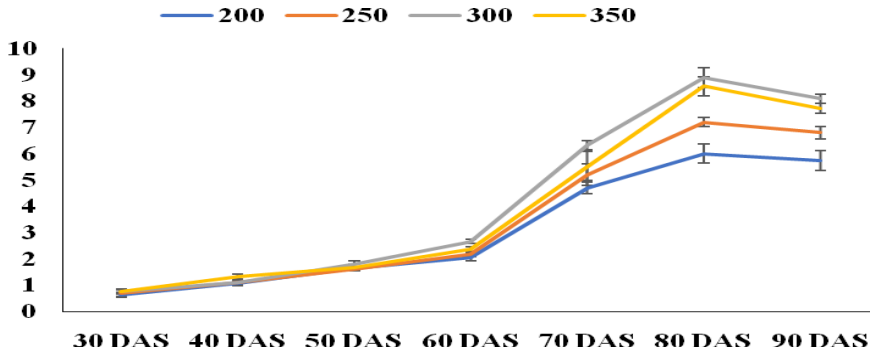


Figure 2: Dry weight of BAU chia-1 at different growth stages under different seed rate levels. The vertical bars represent the standard error. Here, T_1 = 200 g ha^{-1}, T_2 = 250 g ha^{-1}, T_3 = 300 g ha^{-1}, and T_4 = 350 g ha^{-1}.

Leaf number plant^{-1}

Table 2 indicates a relationship between seed rate and the average number of leaves per plant. As the seed rate increases, the leaf number plant^{-1} increases from approximately 41 to 54. The highest leaf number (54) was observed at the highest seed rate (T_4). Conversely, the lowest leaf number (41) was recorded at the lowest seed rate (T_1). These findings align with common agronomic observations where optimal plant spacing enhances individual plant performance. Lower seed rates of T_1 may result in competition with weeds for available light, nutrients, and water resources (Holdsworth, 1956; Bernier *et al.,* 2018). This competition decreases the plant height, node number, and ultimately the leaf number in an individual plant.

Branch starting at the Nodal Position: Table 2 showed that branching began at 3 to 4 nodes across the treatments, indicating a moderate impact of seed rate on the timing of branching. At lower seed rates (T_1 and T_2), branching initiated at the 3rd node, whereas in higher seed rates (T_3 and T_4), branching starts with the 4th node. This slight delay in branch initiation at higher seed rates may be due to increased inter-plant competition. This competition delays the expression of secondary structures like branches. However, denser populations possibly prioritize vertical growth and leaf expansion before branching begins (Biswas *et al.,* 2020; Bennett *et al.,*2012).

Canopy spread: The canopy spread showed a consistent decrease with increasing seed rate, reflecting a pattern similar to that observed in leaf number and branch initiation (Table 2). The widest canopy spread (44.67 cm) was recorded at the lowest seed rate (T_1), while the narrowest spread (35.33 cm) was at the highest seed rate (T_4). Plants grown at lower rates benefit from more available resources, which supports the broader canopy development (Biswas *et al.,* 2020). A wider canopy allows for better light interception and may contribute to enhanced photosynthetic capacity, potentially improving biomass accumulation and yield. Conversely, increased plant density leads to reduced lateral growth, thus restricting canopy spread. This may limit light penetration, reduce the photosynthetic efficiency of lower leaves, possibly affecting crop performance (Huber *et al.,* 2021).

Table 1: Effect of seed rates on leaf number plant^{-1}, starting branches at the nodal position, and canopy spread of BAU chia 1 at different days after sowing (DAS)

Treatment	Leaf number plant^{-1}	Starting branches at the nodal position (count from the base)	Canopy spread (cm)
T_1	41^{c}	3^{c}	44.67^{a}
T_2	45^{bc}	3^{b}	40.33^{b}
T_3	50^{ab}	4^{a}	37.33^{bc}
T_4	54^{a}	4^{a}	35.33^{c}
% CV	11.66	16.49	12.66

In a column, figures with the same letter do not differ significantly. Here, T_1 = 200 g ha^{-1}, T_2 = 250 g ha^{-1}, T_3 = 300 g ha^{-1}, and T_4 = 350 g ha^{-1}.

Photosynthetically Active Radiation Efficiency: A clear increase in photosynthetically active radiation (PAR) was shown in Figure 2. PAR progressively increased across all treatments starting from 30 DAS. At 70 DAS, it peaked in all treatments, then slightly declined, as observed at 90 DAS. Among the treatments, T_4 consistently recorded the highest PAR at nearly all stages, followed by T_3. At 70 DAS, PAR of T_4 was 87.48%, followed by T_3 (86.51%), T_2 (84.23%), and T_1 (82.89%). On the other hand, T_1 exhibited the lowest PAR throughout the growth period. This suggests that increasing the seed rate positively influenced PAR efficiency and biomass accumulation up to a certain period. It is reasonable since land was covered by leaves (full canopy) and received maximum light (Pei *et al.,* 2022). However, the decline in PAR efficiency may be due to leaf senescence and canopy aging (Li *et al.,* 2022). The marginal difference between T_3 and T_4 indicates that both optimize PAR efficiency in the chia field.

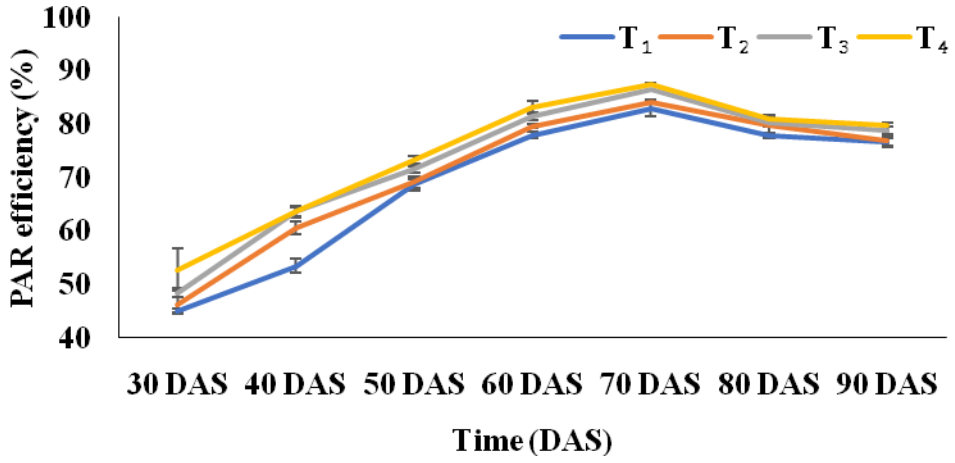


Figure 3: Photosynthetically Active Radiation efficiency of BAU chia-1 at different growth stages under different seed rate levels. The vertical bars represent the standard error. Here, T_1 = 200 g ha^{-1}, T_2 = 250 g ha^{-1}, T_3 = 300 g ha^{-1}, and T_4 = 350 g ha^{-1}.

Inflorescence number plant^{-1}

The inflorescence number plant^{-1} ranged from 6 to 8, as shown in Table 3. The highest value was observed in T_1 (8), indicating better reproductive development. T_3 and T_4 showed fewer inflorescences (6), and T_2 showed 7 inflorescences plant^{-1}. The highest inflorescence number in T_1 indicates more favorable growing conditions, such as lower plant competition or better nutrient availability. On the other hand, the higher plant density or limited resources might have suppressed reproductive branching. A higher number of inflorescences is generally advantageous, contributing to more sites for seed production (LeHeiget *et al.,* 2023).

Main inflorescence length (cm)

Table 3 shows that the main inflorescence length varied from 11.1 cm to 14.83 cm. T_2 showed superior performance, which could be attributed to optimal plant spacing or resource availability. A longer main inflorescence can accommodate more spikelets and ultimately, more seeds, contributing positively to yield (LeHeiget *et al.,* 2023). Shorter lengths in T_4 suggest possible limitations in growth due to a lack of nutrients or other factors.

Spikes main inflorescence^{-1}

The number of spikes on the main inflorescence varied across treatments. The highest number of spikes (18) was observed in T_2, followed by T_1 (17). T_3 and T_4 treatments recorded lower values, with 15 and 14 spikes, respectively. The decline in spike number in T_3 and T_4 could be due to excessive plant population, which may hinder spike initiation and development.

Spikelets spike^{-1}

Spikelets spike^{-1} ranged from 5.33 in T_4 to 7.67 in T_1. A higher number of spikelets indicates a better potential for seed. T_1 showed the highest value, slightly outperforming T_2. Lower spikelet counts in T_3 and T_4 suggest that denser planting or suboptimal growing conditions may have restricted spike development.

Table 2: Effect of seed rates on inflorescence number plant^{-1}, main inflorescence length, spikes main inflorescence^{-1}, and spikelet spike^{-1} of chia at different days after sowing (DAS)

Treatment	Inflorescence number plant^{-1}	Main inflorescence length (cm)	Spikes main inflorescence^{-1}	Spikelet spike^{-1}
T_1	8^{a}	13.43^{ab}	17^{ab}	7^{a}
T_2	7^{a}	14.83^{a}	18^{a}	7^{a}
T_3	6^{a}	12.90^{ab}	15^{bc}	6^{ab}
T_4	6^{a}	11.10^{b}	14^{c}	5^{b}
% CV	12.66	11.82	11.18	15.81

In a column, figures with the same letter do not differ significantly. Here, T_1 = 200 g ha^{-1}, T_2 = 250 g ha^{-1}, T_3 = 300 g ha^{-1}, and T_4 = 350 g ha^{-1}.

Seed yield

The seed yield varied noticeably among treatments, as depicted in Figure 3. The highest yield (1116.67 kg ha^{-1}) was observed in T_2, followed by T_3 (1083.33 kg ha^{-1}). The lowest yield was recorded in T_1 (883.33 kg ha^{-1}). This indicates that moderate seed rates yielded better

outcomes. Despite T_1 showing superior vegetative growth, its lower yield suggests that vegetative vigor alone does not assure higher productivity. However, T_4 showed a higher yield than T_1 but still underperformed compared to T_2 and T_3. This may be due to increased competition at high plant densities, limiting individual plant performance.

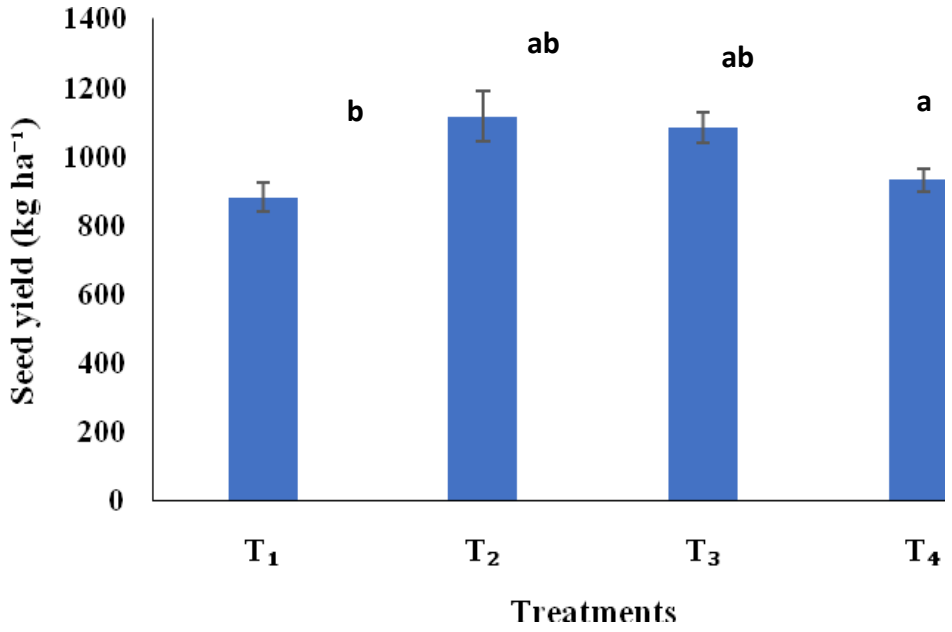


Figure 4: Seed yield of BAU Chia 1 at different seed rate levels. The vertical bars represent the standard error. Here, T_1 = 200 g ha^{-1}, T_2 = 250 g ha^{-1}, T_3 = 300 g ha^{-1}, and T_4 = 350 g ha^{-1}.

Conclusion

This study demonstrates the profound impact of seed rate on both vegetative and reproductive growth parameters, ultimately influencing seed yield. Four seed rates were evaluated, and different vegetative and reproductive parameters were assessed. The study highlighted that a 250 g ha^{-1} seed rate is the most effective in achieving optimal growth and seed yield as well. However, excessive seed rate (350 g ha^{-1}) is not ideal for maximizing yield. Instead, as seen in 250 g ha^{-1} (optimal seed rate), a balanced approach ensures optimal resource utilization, promotes efficient reproductive development, and enhances overall productivity. Finally, it might be helpful for seed rate recommendations and improving yield outcomes. Multilocation trials are needed to confirm the findings.

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