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RESEARCH ARTICLE

Evaluation of sugar beet growth indices affected by biological fertilizer and nitrogen under delayed planting conditions

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Abstract

Under delayed planting conditions in sugar beet (*Beta vulgaris* L.), integrating nitrogen fertiliser with plant growth-promoting rhizobacteria (PGPR) can enhance plant development and partially compensate for the reduced growing season. To evaluate this interaction, a split-split plot experiment was conducted with three replications and eight treatments. The main factor was planting date with two levels: normal planting (D_1) and delayed planting (D_2). Sub-plots consisted of nitrogen fertiliser at two levels: the recommended rate (N_1; 300 kg urea ha^{-1}, based on local fertiliser guidelines) and 25 % less than the recommended rate (N_2). Sub-sub-plots included bacterial inoculation treatments: with PGPR inoculation (B_2) and without inoculation (B_1). Growth indices, including leaf area index (LAI), total dry matter (TDM), crop growth rate (CGR), relative growth rate (RGR) and net assimilation rate (NAR), were measured. The highest LAI (3.5), total dry matter accumulation [2898 g m^{-2} in growing degree days (GDD)] and maximum CGR, RGR and NAR were recorded in both planting dates under the combined treatment of recommended nitrogen fertiliser (N_1) and PGPR inoculation (B_2). Conversely, the lowest values for all growth parameters were obtained in treatments with reduced nitrogen (N_2) and no bacterial inoculation (B_1), regardless of planting date. Statistical analysis indicated that differences among treatments were significant ($p < 0.05$). All measured growth indicators were significantly improved compared to the reduced nitrogen without inoculation treatment, with increases ranging from 21–43 % depending on the parameter. These findings demonstrate that integrating chemical and biological fertilisers is an effective strategy to mitigate the adverse effects of delayed sowing and improve sugar beet growth performance.

Keywords: growth rate; leaf area index; nitrogen fertiliser; planting date; plant growth-promoting rhizobacteria; sugar beet

Introduction

The global demand for sweet foods is widespread and continuously increasing. Sugar beet (*Beta vulgaris* L.) is the second most important source of sugar worldwide, playing a vital role in the sugar industry of many countries (1). As a major biennial crop harvested in its first year for sugar production, sugar beet requires a relatively long growth period, typically ranging from 140 to 200 days (2). Suboptimal planting schedules and imbalanced fertiliser application are major causes of reduced root yield and sugar content in sugar beet. Planting date is a critical management factor significantly influencing yield and other agronomic traits (3). Delayed planting often shortens the growing season, leading to yield reduction, whereas early planting tends to extend the growth duration, which is a key factor influencing yield variation in sugar beet (4).

Nitrogen is a critical macronutrient for sugar beet growth, yield and quality, serving as a major limiting factor for production. Nitrogen deficiency restricts yield more than any other nutrient (5). While conventional agriculture primarily relies on chemical nitrogen fertilisers, their appropriate use is crucial for improving crop productivity and enhancing nitrogen use efficiency (NUE) (5). Globally, research has explored alternative strategies to compensate for nitrogen deficiency, such as seed inoculation with beneficial microorganisms like *Azotobacter*, *Azospirillium* and *Pseudomonas* (6). Biofertilisers, containing one or more plant growth-promoting bacteria, are vital for maintaining soil biological balance, optimising agroecosystem interactions and improving soil physico-chemical properties (7). Seed inoculation with plant growth-promoting rhizobacteria (PGPR) is an effective strategy to enhance plant performance. PGPR establish beneficial associations with plant roots, improving growth and yield through various mechanisms, including enhanced stress tolerance, biological nitrogen fixation, solubilization of inorganic phosphate and other minerals, improved nutrient uptake, pathogen inhibition and production of antibiotics and enzymes (8, 9). In sugar beet cultivation, biofertilizer application as a seed treatment has been shown to significantly improve dry weight accumulation, leaf area index (LAI), crop growth rate (CGR) and net assimilation rate (NAR) (10).

However, limited information is available on the combined effects of planting date and the integrated application of chemical and biological fertilisers on sugar beet growth and physiological indices. In many arid and semi-arid regions, water scarcity forces farmers to delay sugar beet planting due to temporal overlap with the final irrigation of preceding cereal crops. This delay constrains the growing season and potentially limits yield. Therefore, this study aimed to identify sustainable agronomic strategies to enhance sugar beet growth under delayed planting conditions. It is hypothesised that the integration of biofertilisers with reduced levels of chemical nitrogen fertilisers can improve nitrogen use efficiency, physiological performance and sugar beet yield under delayed planting conditions.

Materials and Methods

Experimental site and design

This experiment was conducted as a split-split plot arrangement in a randomised complete block design (RCBD) with three replications at the research farm of Ferdowsi University of Mashhad, Iran (36°18′ N latitude, 59°36′ E longitude, altitude ~ 985 m) during the 2021–2022 growing season. The region has a semi-arid climate. The experimental factors included planting date at two levels (D_1: normal and D_2: delayed) as the main plot factor, nitrogen fertilizer (urea) at two levels (N_1: recommended dose of 300 kg ha^{-1}; N_2: 25 % below recommended dose, 225 kg ha^{-1}) as the sub-plot factor and bacterial inoculation at two levels (B_1: no inoculation; B_2: inoculation with PGPR) as the sub-sub-plot factor, resulting in eight treatment combinations.

Cultural practices and treatments

The experiment was carried out using the 'Arta' sugar beet cultivar. Each plot consisted of six planting rows, each 5 m in length, with a row spacing of 50 cm and an intra-row spacing of 20 cm. A 50 cm buffer zone was maintained between sub-plots and a 1 m buffer between blocks to prevent cross-contamination. Soil samples were collected before planting and analysed (Table 1). A commercial PGPR product (biofertilisers, containing a consortium of *Azotobacter chroococcum* and *Azospirillium brasilense* strains, produced by Royan Tisan Co., Iran, with a viable count of 1×10^9 CFU mL^{-1}, formulated in a peat-based carrier) was applied at a rate of 5 L ha^{-1} in two stages: as a seed inoculant before planting and during the first irrigation after planting. Urea fertiliser was applied in two splits: at the six-leaf stage and two weeks later. The recommended nitrogen rate (300 kg urea ha^{-1} was determined based on local soil test recommendations (11).

Data collection

Starting at the six-leaf stage, eight plants were randomly sampled from the two central rows of each sub-plot every two weeks. Sampling was performed 8 times for the normal planting date and 8 times for the delayed planting due to the shorter growing season. Plants were separated into roots, leaves and petioles. Leaf area was measured using an LI-3100C leaf area meter (LI-COR Biosciences, USA). The LAI was calculated as the total leaf area per unit ground area. Samples were dried at 80 °C for 72 hr and dry weights of plant components were measured using a digital balance (accuracy 0.01 g). Data were scaled to represent values per square meter.

Growth indices and thermal time

The relationship between dry weight (Y) and accumulated thermal time (X) was modelled using a sigmoid function (Equation 1). Thermal time was calculated as the sum of $((T_{max} + T_{min})/2)$ - Tb, where T_{max} and T_{min} are daily maximum and minimum temperatures, Tb is the base temperature (3 °C for sugar beet) and n is the number of days after planting.

$$Y = a / [1 + b \times \exp(c \times X)] \quad \text{(Eqn. 1)}$$

Coefficients a, b and c were estimated for each treatment using slide write 4.1 software. Crop growth rate (CGR) was derived by differentiating the equation 1, 2. Relative growth rate (RGR) and net assimilation rate (NAR) were calculated using equation 3, 4.

$$CGR = [a \times b \times c \times \exp(c \times X)] / [1 + b \times \exp(c \times X)]^2 \quad \text{(Eqn. 2)}$$

$$RGR = CGR / TDM \quad \text{(Eqn. 3)}$$

$$NAR = CGR / LAI \quad \text{(Eqn. 4)}$$

Where TDM is total dry matter and LAI is leaf area index.

Statistical analysis

Data were processed using Excel 2016 and Minitab 17. Analysis of variance (ANOVA) was performed using the general linear model in Minitab 17, considering the split-split plot structure of the experiment. The significance of differences between treatment means was compared using Fisher's least significant difference (LSD) test at a 5 % probability level ($p \leq 0.05$).

Result and Discussion

Leaf area index (LAI)

Changes in LAI followed a sigmoidal pattern across all treatments (Fig. 1). LAI remained low during early growth (up to ~ 1000 GDD) due to small leaf size and incomplete canopy closure, then increased rapidly to a maximum before declining gradually due to leaf senescence. A significant difference ($p < 0.05$) was observed between planting dates. Under normal planting, maximum LAI was 3.3 at 3822 GDD, whereas under delayed planting, it was slightly higher (3.4) but occurred earlier, at 2586 GDD (Fig. 1). The higher ambient temperature during delayed planting likely accelerated leaf expansion. Application of the recommended nitrogen rate combined with PGPR inoculation significantly ($p < 0.01$) increased LAI (Fig. 2, 3). The highest LAI (3.05) was obtained from the N_1B_2 treatment, while the lowest value occurred in the N_1B_1 treatment (Fig. 3). This improvement is attributed to the role of PGPR in enhancing nutrient uptake and stimulating the production of growth hormones like auxins and cytokinins, which promote cell division and expansion (12, 13). These results align with previous findings that integrated nutrient management improves vegetative growth and photosynthetic efficiency in sugar beet (14).

Dry matter accumulation (TDM)

Total dry matter accumulation exhibited a sigmoidal trend across all treatments (Fig. 4–6). Initially, TDM accumulation was low due to the limited photosynthetic area. As the canopy developed and LAI increased, photosynthetic activity intensified, leading to a sharp rise in dry matter. The rate of accumulation gradually plateaued later in the season due to leaf senescence and increased respiration.

Table 1. Some characteristics of the farm soil of Ferdowsi University of Mashhad, Faculty of Agriculture

pH	$EC_{ds/m}$	N %	P mg/kg	K mg/kg	OC %	OM %	Texture
8.11	0.85	72	12.5	387	0.85	1.45	Sand clay loam

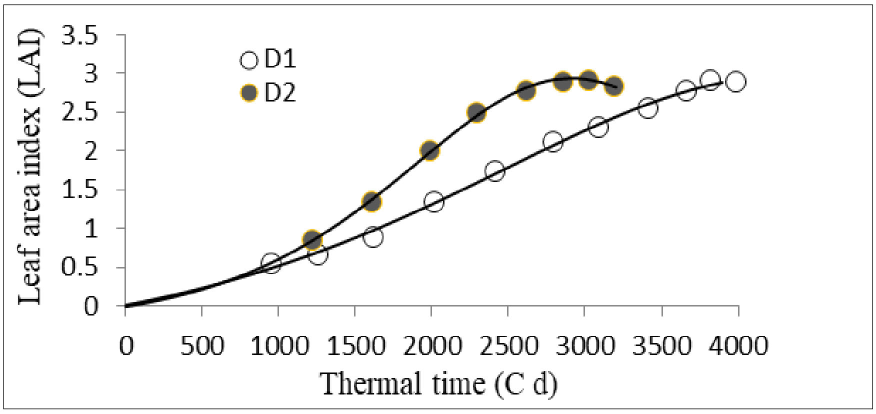

Fig. 1. Comparison of leaf area index (LAI) between two planting dates: D_1 (normal planting date) and D_2 (delayed planting date).

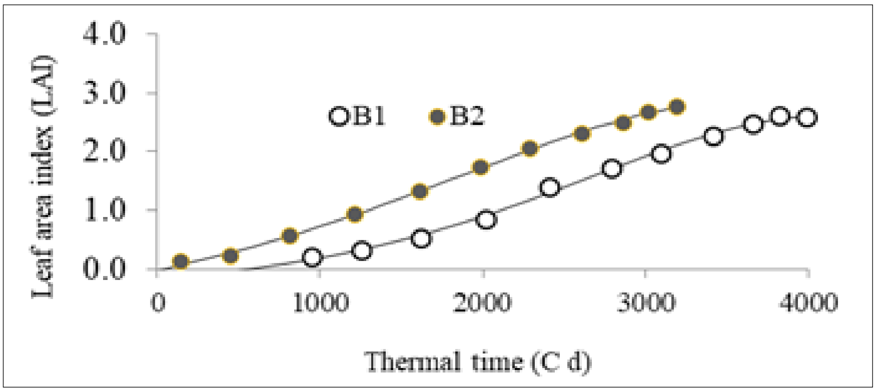

Fig. 2. Effects of growth-promoting bacteria on leaf area index (LAI). Comparison between non-inoculated treatment (B_1) and inoculated treatment (B_2) with growth-promoting bacteria.

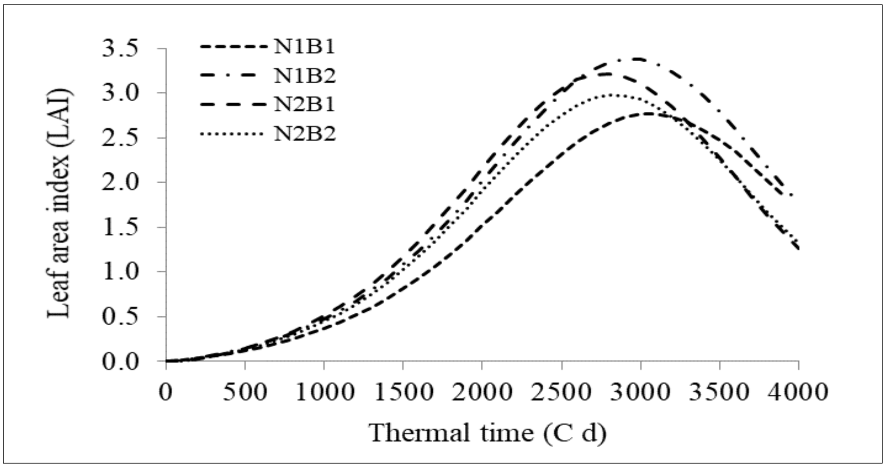

Fig. 3. Comparison of leaf area index (LAI) among nitrogen fertiliser and bacterial inoculation treatments: recommended nitrogen without bacteria inoculation (N_1B_1), recommended nitrogen with bacteria inoculation (N_1B_2), 25 % less than recommended nitrogen without bacteria inoculation (N_2B_1) and 25 % less than recommended nitrogen with bacteria inoculation (N_2B_2).

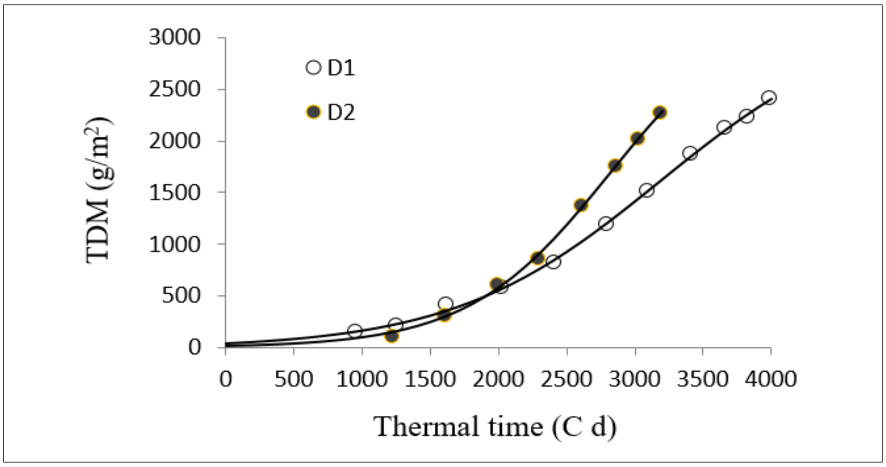

Fig. 4. Comparison of total dry matter accumulation (TDM) between two planting dates: D_1 (normal planting date) and D_2 (delayed planting date).

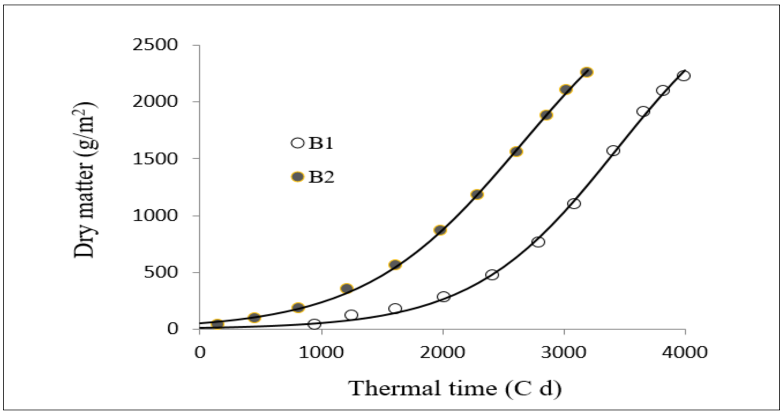

Fig. 5. Comparison of total dry matter accumulation (TDM) between treatments without growth-promoting bacteria (B_1) and with bacterial inoculation (B_2).

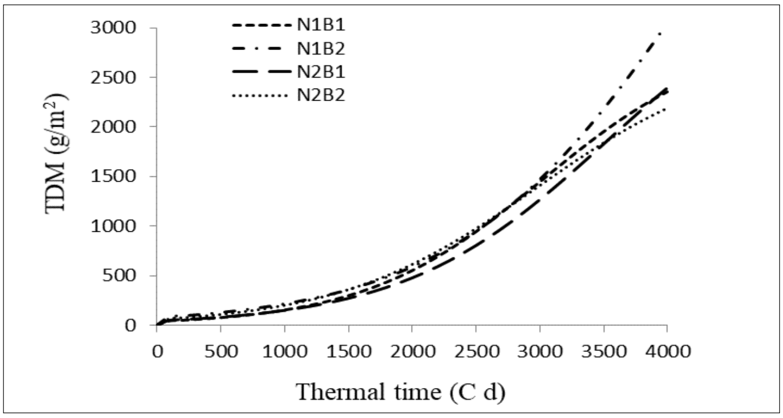

Fig. 6. Comparison of cumulative total dry matter (TDM) during the growing season among treatments: nitrogen fertilizer based on recommendation without bacterial inoculation (N1B1), nitrogen fertilizer based on recommendation with bacterial inoculation (N_1B_2), nitrogen fertilizer 25 % below recommendation without bacterial inoculation (N_2B_1) and nitrogen fertilizer 25 % below recommendation with bacterial inoculation (N_2B_2).

The trend of TDM closely mirrored that of LAI (Fig. 1–6). A linear increase in TDM began at approximately 1200 GDD for normal planting and 1500 GDD for delayed planting. The delayed planting exhibited a more linear accumulation pattern, likely because higher temperatures accelerated thermal unit accumulation, shortening the vegetative phase (15). Nitrogen fertiliser application combined with bacterial inoculation significantly enhanced dry matter accumulation (Fig. 5, 6). The highest mean TDM (2983 ± 105 g m^{-2}) was recorded at 3990 GDD under the N_1B_2 treatment, while the lowest TDM (2089 ± 94 g m^{-2}) occurred at 3189 GDD in the N_2B_1 treatment (Fig. 6). The positive impact of PGPR is mainly attributed to improved nutrient availability and production of growth-regulating substances, which stimulate vegetative growth and enhance assimilate partitioning (16, 17). These findings are consistent with reports demonstrating that integrating nitrogen fertilisation and PGPR significantly improves biomass accumulation in sugar beet (17, 18).

Crop growth rate (CGR)

The pattern of CGR variation followed a sigmoidal curve for both planting dates (Fig. 7). Low LAI during early crop development restricted light interception and photosynthetic activity, resulting in low CGR. As the canopy expanded, enhanced light absorption and photosynthetic efficiency led to a rapid increase in CGR. Notable differences were observed between planting dates. The maximum CGR under delayed planting reached 1.7 g m^{-2} °C^{-1} day^{-1}, whereas under normal planting, it was 1.0 g m^{-2} °C^{-1} day^{-1} (Fig. 7). The more pronounced increase under delayed planting can be attributed to higher temperatures accelerating canopy growth earlier in the season. As thermal time advanced, leaf senescence

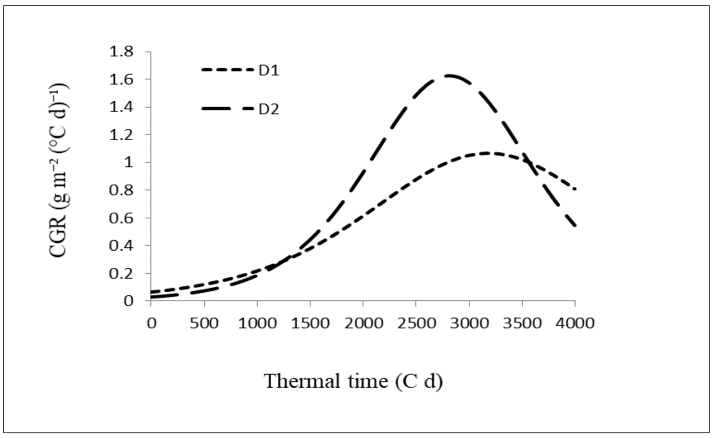

Fig. 7. Comparison of crop growth rate (CGR) in (D_1) normal planting date and (D_2) delayed planting date.

and a decline in net photosynthetic rate reduced CGR. Nitrogen fertilisation combined with PGPR inoculation significantly influenced CGR (Fig. 8, 9). The highest mean CGR (1.8 ± 0.06 g m^{-2} °C^{-1} day^{-1}) was recorded in the N_1B_2 treatment under normal planting conditions. Conversely, the lowest CGR (1.4 ± 0.05 g m^{-2} °C^{-1} day^{-1}) was observed in the N_2B_1 treatment (Fig. 9) (19, 20).

Relative growth rate (RGR)

Analysis of RGR across planting dates revealed three distinct growth stages (Fig. 10). In normal planting, the first stage lasted up to ~1800 GDD, while in delayed planting, this phase extended only to ~1500 GDD. This initial phase was characterised by low LAI (0.8–1.2), minimal canopy shading and a small proportion of non-photosynthetic tissues. The second stage was characterised by a linear decline in RGR, likely associated with a higher allocation of assimilates to root and structural tissues rather than leaf expansion. This phase continued until ~3800 GDD in normal planting and ~3500 GDD in delayed planting. In the third stage, RGR continued to decline but at a slower rate, coinciding with LAI reduction due to leaf senescence. The recommended nitrogen rate combined with PGPR inoculation produced the highest mean RGR among treatments (~0.085 g g^{-1} °C^{-1} day^{-1}), while the lowest RGR (~0.064 g g^{-1} °C^{-1} day^{-1}) occurred in the N_2B_1 treatment (Fig. 11, 12). These results demonstrate that integrated nutrient management with bacterial inoculation effectively maintains higher relative growth rates, enhancing overall biomass accumulation (21, 22).

Net assimilation rate (NAR)

Throughout the growing season, LAI and leaf dry weight increased, but a significant portion of leaf biomass consisted of structural or storage tissues with reduced photosynthetic activity. Initially, expanding LAI enhanced canopy photosynthesis, yet mutual shading limited light interception in lower leaves, reducing their contribution and causing NAR to decline. A slight recovery occurred later due to the senescence of older leaves. The decline in NAR was faster under delayed planting, reflecting accelerated canopy development.

The temporal pattern of NAR showed three phases: Phase 1 (early season, 0–1000 GDD normal; 0–800 GDD delayed) with low LAI and TDM; Phase 2 (1000–2500 GDD normal; 800–2200 GDD delayed) with a gradual NAR increase as LAI rose; and Phase 3 (2500–end; 2200–end delayed) with a final NAR decline due to senescence (Fig. 13). The highest NAR was observed in the N_1B_2 treatments, reflecting improved aerial growth and photosynthetic efficiency, while reduced nitrogen or no inoculation resulted in lower NAR, as shown in Fig. 14, 15 (10, 23, 24).

Conclusion

The findings of this study confirm the initial hypothesis that the combined application of PGPR with nitrogen fertiliser at the recommended rate can effectively enhance sugar beet growth and physiological performance under delayed planting conditions. All measured growth indicators, including LAI, TDM, CGR, RGR and NAR, were significantly improved compared to the treatment with 25 % reduced nitrogen and no inoculation, with increases ranging from 21 % to 43 %. This integrated approach not only mitigates the negative effects of a shortened growing season but also improves nitrogen use efficiency, potentially reducing environmental pollution by minimising nitrate leaching. This strategy offers a sustainable agronomic practice for sugar beet production in arid and semi-arid regions where delayed planting is common. Considering the study was conducted in a single location and season, further research across different regions, seasons and with various PGPR strains is recommended to validate these findings. Future studies incorporating economic analysis are also warranted to establish the optimal economic dose of nitrogen when combined with PGPR.

Acknowledgements

We would like to express our heartfelt gratitude to the experimental farm staff, whose valuable contributions greatly supported the implementation of this experiment.

Authors' contributions

NN conducted the research and prepared the initial draft of the manuscript. WAS reviewed the manuscript and revised it for scientific content. SS contributed to data collection and data analysis. All authors read and approved the final manuscript.

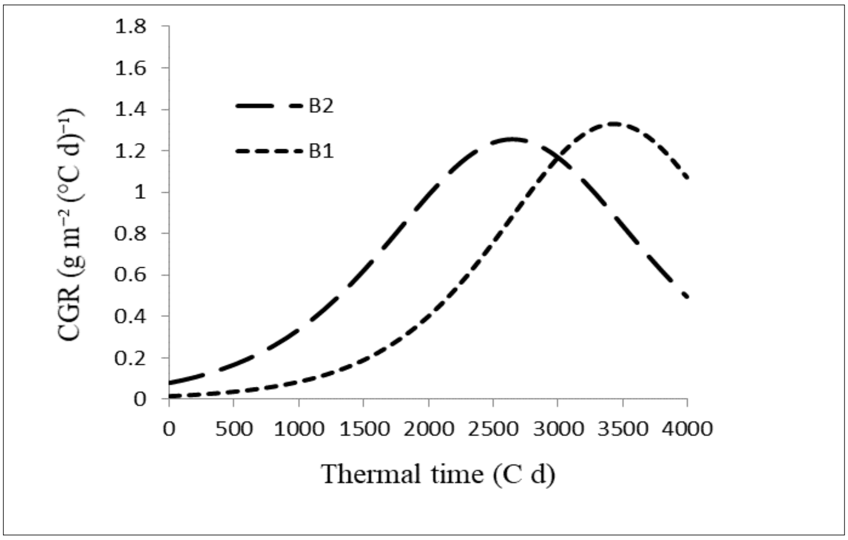

Fig. 8. Comparison of crop growth rate (CGR) under two treatments: (B_1) without growth-promoting bacteria and (B_2) with inoculation of growth-promoting bacteria. Inoculated plants (B_2) exhibited higher CGR, indicating the positive effect of beneficial bacteria on biomass accumulation.

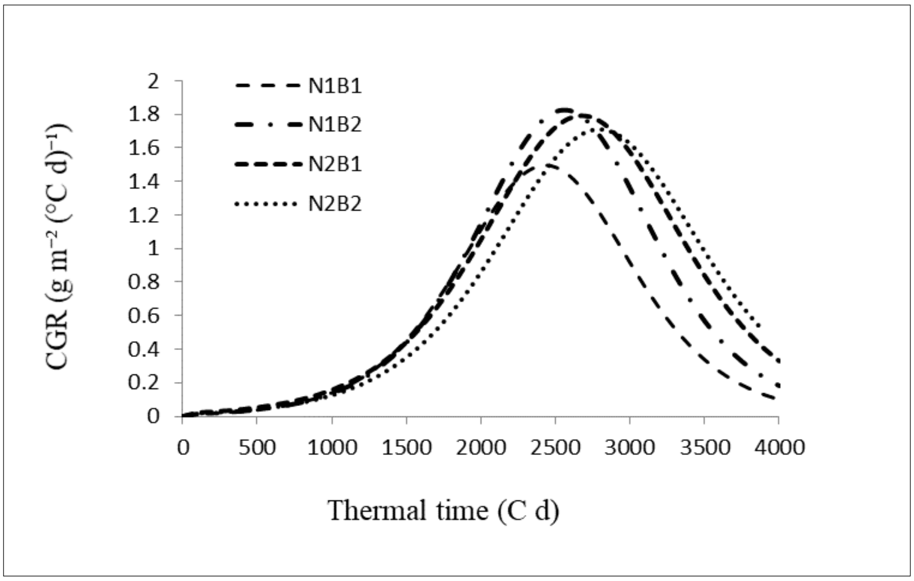

Fig. 9. Comparison of crop growth rate (CGR) during the growing season under different nitrogen fertiliser and bacterial inoculation treatments: (N_1B_1) recommended nitrogen fertiliser without bacterial inoculation, (N_1B_2) recommended nitrogen fertiliser with bacterial inoculation, (N_2B_1) 25 % reduced nitrogen fertiliser without bacterial inoculation and (N_2B_2) 25 % reduced nitrogen fertiliser with bacterial inoculation. The interaction between nitrogen level and bacterial inoculation significantly influenced CGR patterns throughout the growth period.

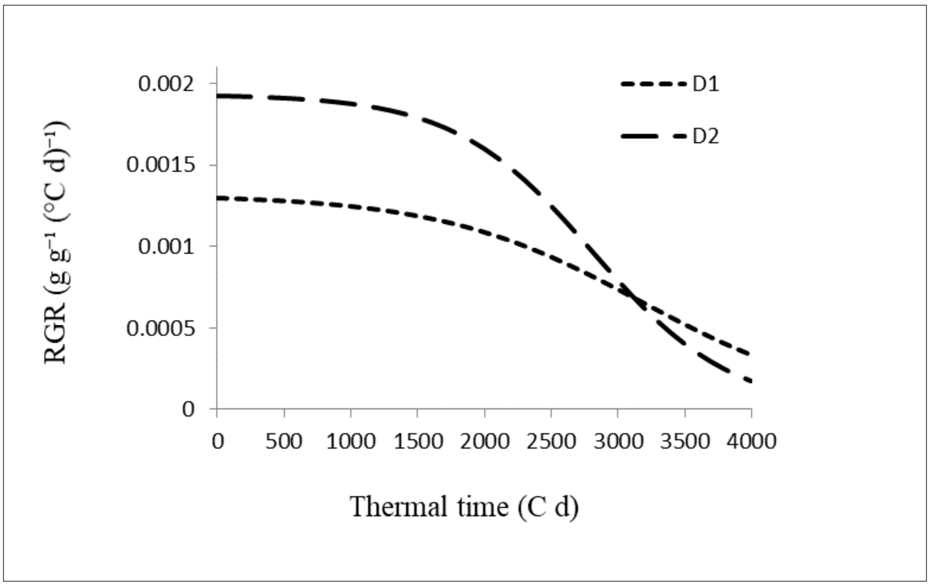

Fig. 10. Comparison of relative growth rate (RGR) in (D_1) normal planting date and (D_2) delayed planting date.

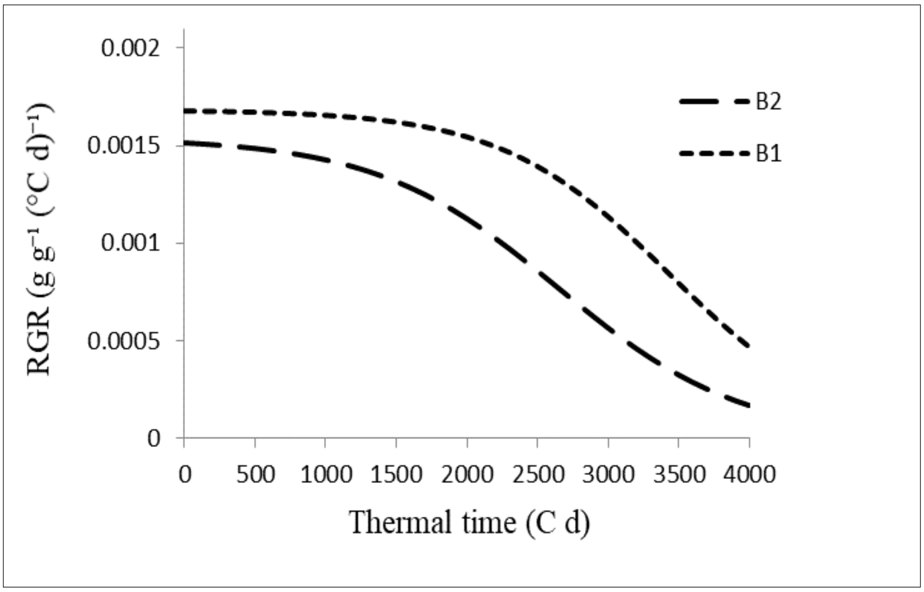

Fig. 11. Comparison of relative growth rate (RGR) in treatment (B_1) without growth-promoting bacteria and (B_2) inoculation with growth-promoting bacteria.

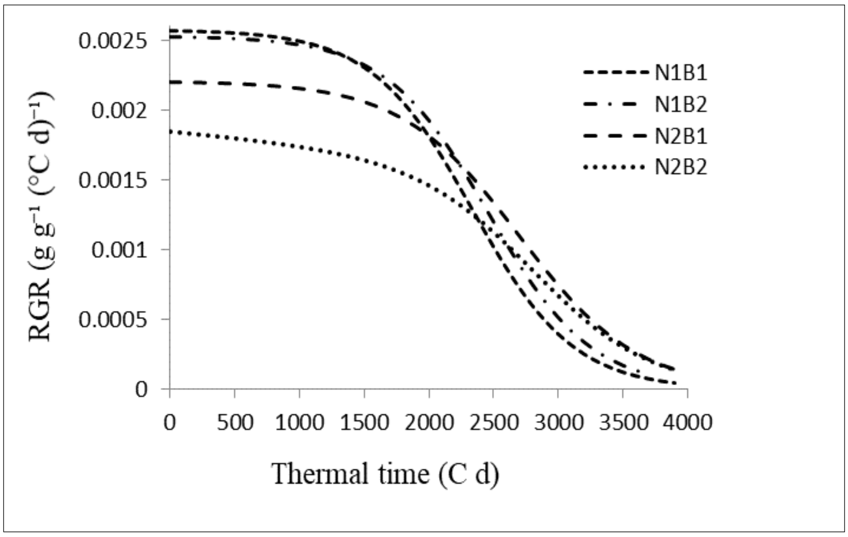

Fig. 12. Relative growth rate (RGR) during the growing season under different nitrogen fertiliser and bacterial inoculation treatments: (N_1B_1) recommended nitrogen fertiliser without bacterial inoculation, (N_1B_2) recommended nitrogen fertiliser with bacterial inoculation, (N_2B_1) 25 % reduced nitrogen fertiliser without bacterial inoculation, and (N_2B_2) 25 % reduced nitrogen fertiliser with bacterial inoculation. The combination of recommended nitrogen and bacterial inoculation (N_1B_2) resulted in the highest RGR, while the lowest RGR was observed in the reduced nitrogen treatment without bacterial inoculation (N_2B_1).

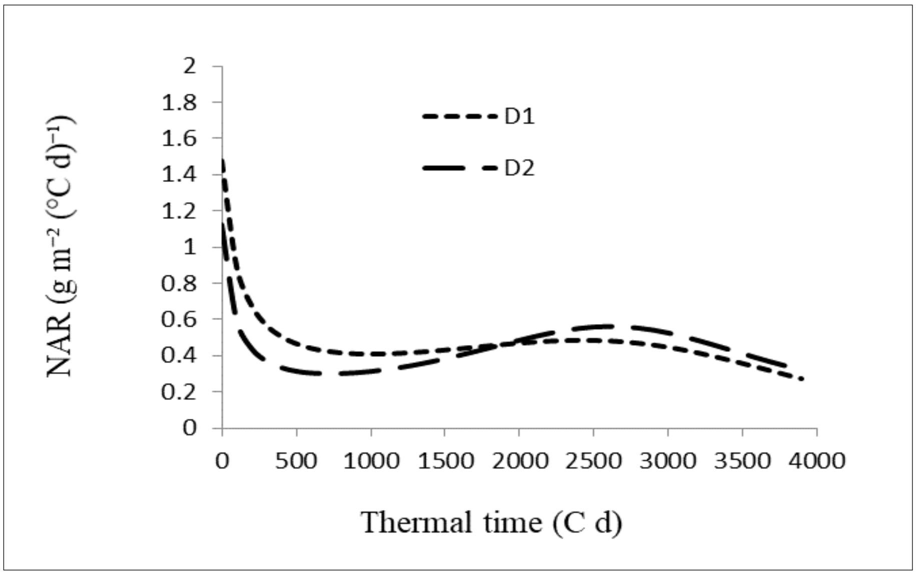

Fig. 13. Comparison of net photosynthesis rate (NAR) in (D_1) normal planting date and (D_2) delayed planting date.

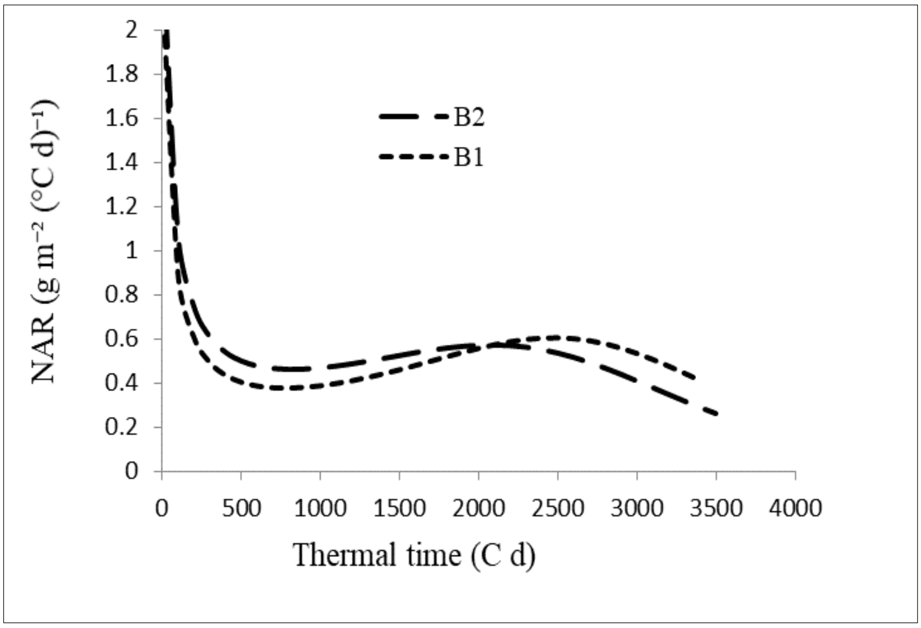

Fig. 14. Comparison of net photosynthesis rate (NAR) in treatment (B_1) without growth-promoting bacteria and (B_2) inoculation with growth-promoting bacteria.

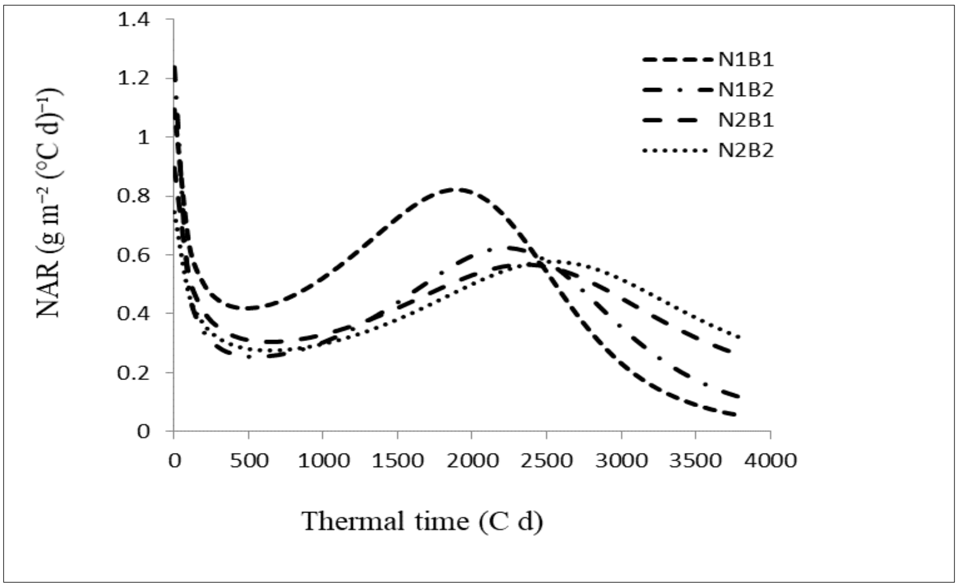

Fig. 15. Net photosynthesis rate (NAR) during the growing season under different treatments: nitrogen fertiliser at recommended rates without bacterial inoculation (N_1B_1), nitrogen fertilizer at recommended rates with bacterial inoculation (N_1B_2), nitrogen fertilizer at 25 % less than recommended without bacterial inoculation (N_2B_1), and nitrogen fertilizer at 25 % less than recommended with bacterial inoculation (N_2B_2).

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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