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Physiology, Growth, and Productivity of Spring–Summer Black Gram (*Vigna mungo* L. Hepper) as Influenced by Heat and Moisture Stresses in Different Dates of Sowing and Nutrient Management Conditions

Purabi Banerjee [1], Visha Kumari Venugopalan [2], Rajib Nath [1], Yusuf S. Althobaiti [3], Ahmed Gaber [4,*], Hatim Al-Yasi [4] and Akbar Hossain [5,*]

[1] Department of Agronomy, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur 741252, India; itsmepurabi1@gmail.com (P.B.); rajibbckv@yahoo.com (R.N.)
[2] Division of Crop Sciences, ICAR-Central Research Institute for Dryland Agriculture, Hyderabad 500059, India; visha.venugopal@gmail.com
[3] Department of Pharmacology and Toxicology, College of Pharmacy, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia; ys.althobaiti@tu.edu.sa
[4] Department of Biology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia; h.alyasi@tu.edu.sa
[5] Bangladesh Wheat and Maize Research Institute (BWMRI) Nashipur, Dinajpur 5200, Bangladesh
* Correspondence: a.gaber@tu.edu.sa (A.G.); akbarhossainwrc@gmail.com (A.H.)

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Abstract: Heat and soil moisture stress account for serious abiotic constraint in black gram (*Vigna mungo* (L.) Hepper) production during spring–summer under Gangetic plains of Eastern India. Concurrence of these two can bring about early completion of phenophases that hampers normal metabolism of legumes by disrupting their defense mechanism, leading to poor seed set. The field experiment was conducted with two different sowing dates as the main plot, soil application of cobalt (Co) as subplots and foliar sprays of potassium (K) and boron (B) either alone or in combination as sub–sub plot treatment in a split–split plot design during spring–summer seasons of 2020 and 2021 with black gram (variety: Pant U 31). The study was aimed at evaluating the impact of sowing time and nutrients application alleviating adversities of abiotic stress during reproductive development of black gram. The March first week sown crop took significantly higher days to complete its life cycle compared to March third week sown one (82.0 vs. 78.2 and 81.8 vs. 78.8). This in turn relatively allowed a broader window for leaf area expansion, flowering, and seed filling in the first crop compared to the second one leading to the attainment of superior yield in the normal sown crop during the consecutive years. Crop growth rate (CGR), net assimilation rate (NAR), pod number per plant, seed yield, and harvest index were significantly higher ($p \leq 0.05$) with soil Co @ 4 kg ha^{-1} and foliar 1.25% K + 0.2% B applications through stress mitigation by stimulating chlorophyll biosynthesis, nitrate reductase activity, proline accumulation, and cell membrane stability, irrespective of the years. Fluctuations in per plant pod number explained about 96 and 94% variations in seed yield through linear regressions in respective years. Optimum sowing date along with soil Co application combined with foliar K+B sprays manifested immense potential to achieve higher black gram production. In addition, this nutrient schedule proved to be efficient enough to promote satisfactory growth and optimum seed yield of late sown blackgram through relief of stress during the spring–summer season.

Keywords: black gram; spring–summer; stress; date of sowing; nutrients; productivity

1. Introduction

The phenological behavior and yield formation of any crop have a strong correspondence with the prevailing weather conditions throughout its growing period irrespective of the region [1]. In this context, rising atmospheric temperature and water scarcity are some of the major constraints for developmental aspects of pulse crops in Eastern India [2]. In this context, global food security in the 21st century is literally at stake owing to the rising heat and drought stress introduced by climate change, particularly in the tropics and subtropics [3]. In particular, the exposure to elevated air temperature well above the optimum during the window of February end to mid-June is extremely severe in terms of crop growth. This season invariably lags in soil moisture reserve due to continuous evapotranspiration accompanied by insufficient and erratic rainfall [4]. Temperature together with moisture stress can lead to various morpho-physiological, reproductive, and biochemical peculiarities in plants, getting in the way of their growth and production [5,6]. In fact, these abiotic stresses appearing at the reproductive phase of plants may pose serious threats to the development of leguminous crops by means of altering crop growth rate (CGR), leaf area index (LAI), net assimilation rate (NAR), leaf chlorophyll content, nitrogen fixation and assimilation, flower production and longevity, pollen fertility and viability, pod set, and seed filling, ultimately culminating into poor production [7,8]. The prevalence of moisture stress accompanied by heat stress is more likely to intensify in the upcoming future [9]. This in turn necessitates switching to more efficient and economical agronomic strategies in crop management, largely mitigating the climatic adversities through reduction of intercellular oxidative damage by triggering enzymatic and non-enzymatic antioxidant defence mechanism, induction of osmotic adjustment by accumulating osmotically active substances such as proline and sustaining the whole photosynthetic activity [10].

The farmers of Eastern India mostly keep their lands fallow after the harvest of summer rice during spring–summer. The inclusion of summer pulse crops such as green gram and black gram in this cropping sequence may be a fantastic way out to sustain the fertility of the soil along with some economic harvest at the end of the season. Black gram (*Vigna mungo* (L.) Hepper) is an important short duration pulse crop typically grown in *kharif* season in India [11,12]. The major setback of choosing the spring–summer season for black gram cultivation is the higher temperature and depletion of soil moisture from flowering onwards, critically affecting its nutrient uptake and production potential [13]. Basically, this crop can tolerate an ambient temperature up to 42 °C [14]. However, it prefers cooler temperature at the vegetative phase along with warmer temperature at the later stages of growth, the optimum temperature for its growth being in the range of 18–30 °C [15], more specifically 22–28 °C [16]. Thus, sowing of black gram crop in optimum time is of prime importance for proper harmony between its vegetative and reproductive phases [17], which eventually determines the optimum yield potential of the crop [18]. Apart from this, black gram being a leguminous crop of indeterminate growth habits tends to face constant competition concerning assimilation partitioning between vegetative and reproductive sinks. Translocation of assimilates to the growing vegetative sinks after the onset of the reproductive phase considerably brings down the production potential of the crop due to severe source limitations [19]. Another notable physiological constraint of black gram is flower and fruit drop [20] due to huge intra-plant competition. Hence, improvement in assimilate production along with delay in senescence of reproductive parts are the major areas to be focussed on regarding black gram cultivation.

A good number of research efforts have revealed the crucial and diverse role of the nutrient elements, i.e., cobalt (Co), potassium (K), and boron (B) in the overall growth of pulse crops under normal as well as stress conditions [21–23]. Cobalt is involved with vital physiological and biochemical functions in plants [24], especially the synthesis of leghaemoglobin protein required for rhizobial activity in legumes and subsequent nitrogen fixation manifesting momentous impact on enzyme systems [25]. Cobalt increases amino acid and anti-oxidant enzymes such as SOD content [26]. Side by side, increment of drought resistance and inhibition of ethylene biosynthesis in legume crops through application of cobalt has also been reported [27]. Potassium functions as a catalytic agent in the activation of various enzymes while facilitating assimilate translocation and maintaining osmoregulation in plants [28]. It also prevents drought-induced accumulation of reactive oxygen species (ROS) [29,30]. Documentation on the role of K in increasing proline content in legume crops has also been found [31]. Boron is associated with sugar transportation, photosynthetic activity, pollen germination, formation of flowers, and seed development of pulse crops [32]. Application of foliar sprays of B significantly mitigates drought stress in legumes [33]. Foliar applications of K and B promote rapid translocation of these nutrients, which is very much pertinent in alleviating abiotic stresses in crop plants especially under late sown conditions [34]. However, exogenous application of nutrients has already been proven as a potent tool to mitigate the deleterious effects of heat stress [35]. Earlier literature has also established the fact that legume crops require sufficient K [36] and B supply [37] to combat the detrimental consequences of hot and dry spells, particularly from the onset of flowering.

However, the development of drought resistance in terms of enhancement in enzymatic activities and photosynthetic pigment concentrations, maintenance of cell membrane stability, and accelerated proline accumulation through enhanced water uptake, nitrogen metabolism, and photosynthetic activity, in addition to reduced transpirational rate and delayed senescence in legume crops, has also been registered through the application of Co [26], K [31], and B [33] separately. Thus, it was hypothesized that soil application of Co and foliar sprays of K and B would be an efficient amalgamation of agronomic management in improving black gram production by ameliorating stress during spring–summer. Against this background, a two-year experiment was framed with the following objectives: (1) to analyze the positive impact of different dates of sowing and those nutrients on phenology, biomass, CGR, LAI, and NAR, (2) to evaluate the effect of different sowing time and nutrient application on leaf physiology and yield, and (3) to find out the appropriate date of sowing and nutrient schedule for optimum growth and production of black gram through alleviation of the prevailing high temperature and moisture stress during spring–summer.

2. Materials and Methods

2.1. Details of Experimental Site

The field experiment on black gram (*Vigna mungo* (L.) Hepper) was carried out during spring–summer seasons of 2020 and 2021 at the ‘A-B’ block District Seed Farm (22°93′ N latitude, 88°53′ E longitude) of Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India. The selected site under study is located at an altitude of 9.75 m above mean sea level in a flat topography. The experimental soil is well-drained Gangetic alluvium (order: inceptisol) with moderate fertility and nearly neutral in reaction (pH: 7.5), categorized under the class of clay loam. The soil was found to be low in organic carbon (0.52%: wet digestion method), available nitrogen (146 kg ha^{-1}: alkaline permanganate-oxidizable), boron (0.49 ppm: azomethine H), and cobalt (9.2 ppm: EDTA extractable), and quite rich in available P_2O_5 (38.5 kg ha^{-1}: Bray’s P) and K_2O (194.7 kg ha^{-1}: NH_4OAC-extractable).

2.2. Weather Conditions

The daily data of meteorological parameters concerning temperature and rainfall at Kalyani, Nadia for the study period (March 2020 to June 2020 and March 2021 to May 2021) were collected from AICRP on Agrometeorology, B.C.K.V., Nadia, West Bengal. The mean maximum and minimum temperature, rainfall received, and relative humidity during different growth stages of black gram encompassing both the dates of sowing in 2020 and 2021 have been presented in Table 1 and Figures 1 and 2.

Table 1. Stage-wise mean maximum and minimum temperature and rainfall during black gram growing period.

Parameter	Year	Temperature (°C)			
		At Sowing	Flowering	Pod Initiation	Maturity
Maximum temperature (°C)	2020	29.1 32.5	36.6 37.1	34.0 35.5	33.5 33.8
	2021	30.2 36.7	36.2 36.5	36.9 37.9	35.9 32.3
Minimum temperature (°C)	2020	15.2 16.8	22.9 23.4	23.7 24.3	25.5 24.6
	2021	19.2 19.5	23.8 24.8	24.6 25.0	26.4 25.6
Rainfall (mm)	2020	7.1 2.7	0.0 0.2	0.0 0.0	24.6 14.2
	2021	0.0 0.0	1.3 2.1	1.9 0.7	6.5 31.3
Maximum relative humidity (%)	2020	91.3 90.8	91.5 90.7	91.0 89.4	90.7 90.1
	2021	89.2 84.8	87.9 86.0	87.3 85.4	86.5 86.4
Minimum relative humidity (%)	2020	50.2 47.1	54.7 54.0	54.1 52.7	59.9 57.2
	2021	34.5 27.4	39.1 35.6	37.7 36.6	44.9 44.1

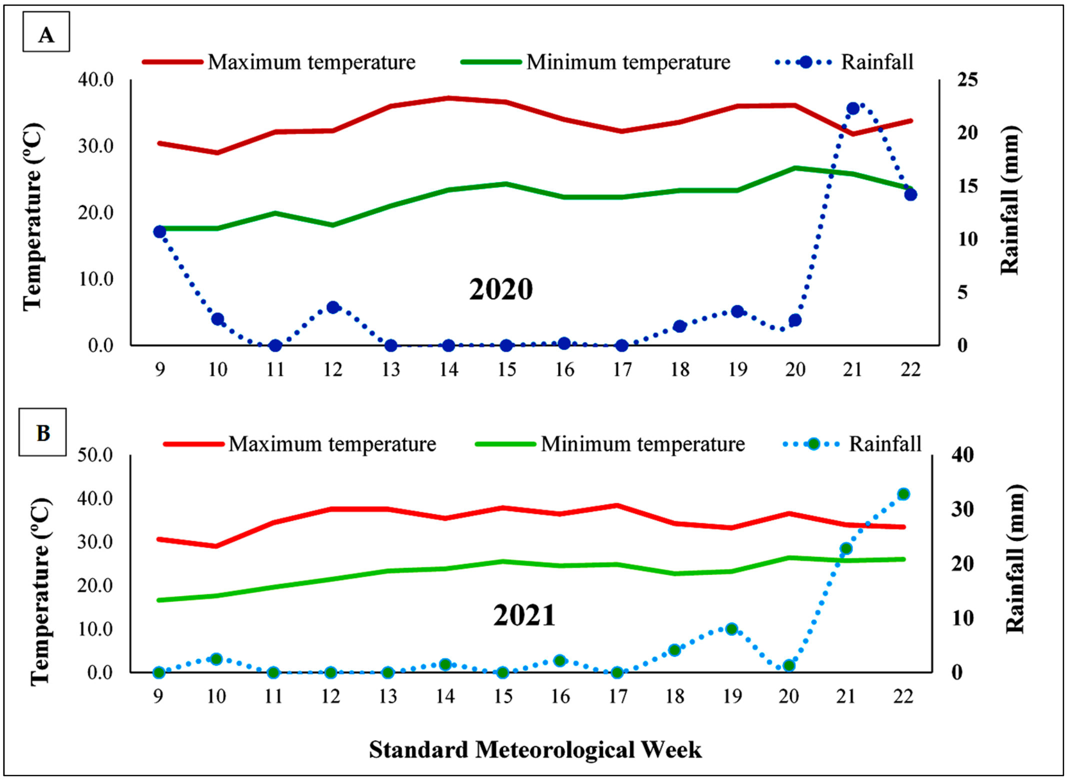

Figure 1. Average weekly distribution of atmospheric temperature and rainfall during black gram growing period: (**A**) 2020 and (**B**) 2021.

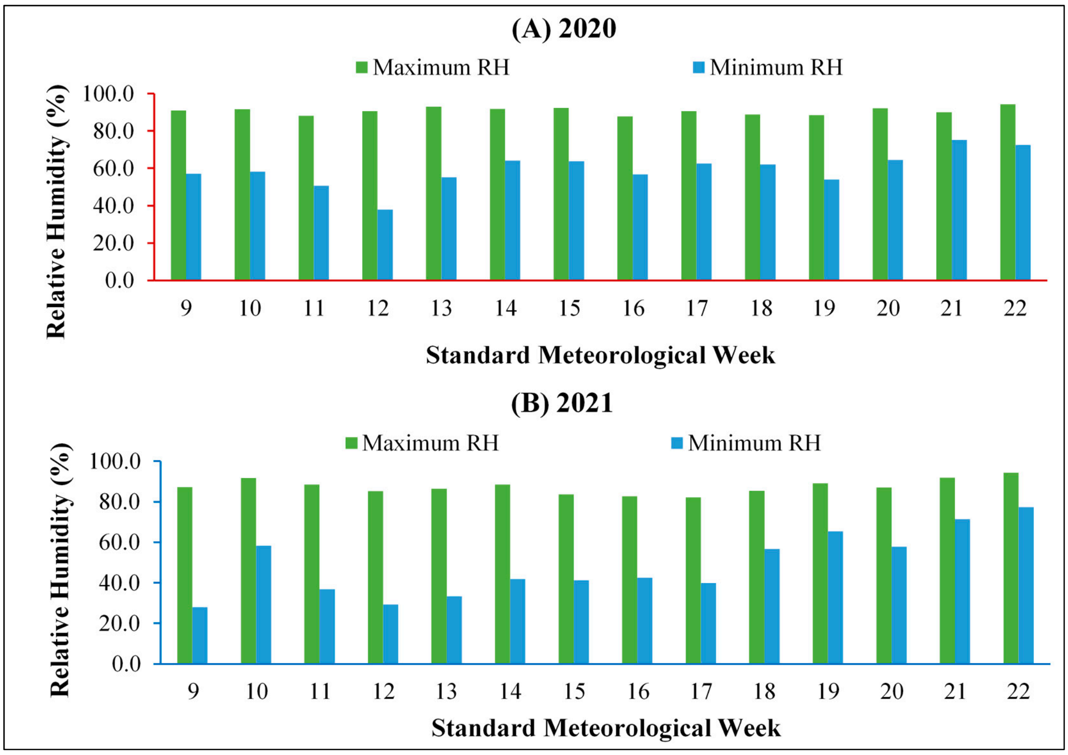

Figure 2. Average weekly distribution of relative humidity during black gram growing period: (**A**) 2020 and (**B**) 2021.

2.3. Experimental Design and Treatment Details

The field experiment was replicated thrice in a split–split plot design. The main plots were comprised of two dates of sowing and subplots were containing two different sets of soil applications of nutrients. Lastly, foliar sprays at the flower initiation stage were applied to sub–sub plots. Detailed descriptions of the treatments are mentioned in Table 2.

Table 2. Treatment details of the field experiment.

Abbreviation	Treatment Description
	Main Plot: Date of sowing (D)
D_1	First week of March (2 March 2020 and 1 March 2021)
D_2	Third week of March (16 March 2020 and 15 March 2021)
	Subplot: Soil application (S)
S_1	No Cobalt application
S_2	Soil application of Co @ 4 kg ha^{-1} (as $Co(NO_3)_2 \cdot 6\ H_2O$ with 20% Co)
	Sub–sub plot: Foliar spray (F)
F_1	No spray
F_2	Foliar spray of tap water @ 500 lit ha^{-1}
F_3	Foliar spray of K @ 1.25% (as Mureate of Potash with 60% K_2O)
F_4	Foliar spray of B @ 0.2% (as Borax with 11.5% B)
F_5	Foliar spray of K @ 1.25% + B @ 0.2%

2.4. Crop Management Practices

Black gram seeds (*var*: Pant U 31) were sown at a row spacing of 30 cm in individual experimental plots of 4 m × 3 m at different dates of sowings. The recommended dose of fertilizers (20:40:40 kg N: P_2O_5: K_2O ha^{-1}) was applied at the time of land preparation prior to seed sowing. One hand weeding was practiced at 25–30 days after sowing in each sowing crop. As the crop faced a bit of rainfall deficit during both spring–summer seasons of 2020 and 2021, every time one pre-sowing irrigation was provided followed by 3 subsequent irrigations at a dry interval of 10 days up to 30 DAS (pre-flowering) for proper stand establishment and nourishment. Foliar sprays with tap water, K, and B were done at flower initiation stage as per the treatment wise nutrient allotments in the morning hours with spraying with the help of a knapsack sprayer by one laborer simply walking along the individual plots.

2.5. Estimation of Crop Growth, Phenology, Physiological Parameters, and Yield Attributes

For taking observations of growth and yield attributes of black gram, 20 plants were randomly selected excluding border row from each plot and were tagged. Each of the phenological stages *viz.*, emergence, branching, flowering, pod initiation, and maturity of black gram sown on different dates were recorded by regular inspection of the experimental field in every two days.

For the estimations of leaf area index (LAI) and total dry matter, samplings were done per plot at vegetative (15–35 DAS), flowering (30–55 DAS), and pod development (45–65 DAS) stages from 10 randomly selected plants. Green leaf portions were separated from the stems for measuring the mean leaf area per plant.

Leaf area index (LAI): LAI was derived using Equation (1) [38]:

$$\mathrm{LAI} = \frac{\text{Leaf area per plant } \left(\mathrm{m}^2\right) \times \text{ Number of plants}}{\text{Ground area } \left(\mathrm{m}^2\right)} \tag{1}$$

Crop growth rate (CGR): Crop growth rate (CGR) indicates the total dry matter production of the crop per unit ground area per unit of time. CGR was expressed in g m^{-2} day^{-1} and was analyzed following Equation (2) [38]:

$$\mathrm{CGR} = \frac{1}{\mathrm{G}} \times \frac{\mathrm{W}_2 - \mathrm{W}_1}{\mathrm{t}_2 - \mathrm{t}_1} \tag{2}$$

where, W_1 = dry weight of the plant (g m^{-2}) at time t_1; W_2 = dry weight of the plant (g m^{-2}) at time t_2; ($t_1 - t_2$) = time interval in days; G = ground area (m^2).

Net assimilation rate (NAR): Net assimilation rate (NAR) is the net gain of assimilates per unit of leaf area per unit time. NAR was worked out using Equation (3) [39], expressed in g m^{-2} leaf area day^{-1}:

$$\mathrm{NAR} = \frac{\log_e \mathrm{L}_2 - \log_e \mathrm{L}_1}{\mathrm{L}_2 - \mathrm{L}_1} \times \frac{\mathrm{W}_2 - \mathrm{W}_1}{\mathrm{t}_2 - \mathrm{t}_1} \tag{3}$$

where L_1 = leaf area (m^2) at time t_1; L_2 = leaf area (m^2) at time t_2; W_1 = dry weight of the plant (g m^{-2}) at time t_1; W_2 = dry weight of the plant (g m^{-2}) at time t_2, ($t_1 - t_2$) = time interval in days; G = ground area (m^2).

Seed and stover yields of black gram were noted at crop maturity.

Harvest index (HI): HI was calculated by using the following Equation (4) [40]:

$$\mathrm{HI}\ (\%) = \frac{\text{Seed yield (kg/ha)}}{\text{Biological yield (kg /ha)}} \times 100 \tag{4}$$

Relative leaf water content (RLWC): To estimate re1ative leaf water content (RLWC), fresh black gram leaves were first collected at 50% flowering stage and cut into smaller pieces. Subsequently, fresh weight (F_w) was noted. In continuation to this, immersion of the leaf cuts in double distilled water was followed for 4 h to obtain the turgid weight (T_w). A constant temperature of 80 ± 1 °C was maintained inside a hot air oven to dry those leaves until a constant dry weight (D_w) was achieved. Relative leaf water content (RLWC) was expressed as the following Equation (5) [41]:

$$\mathrm{RLWC}\ (\%) = \frac{\mathrm{F}_\mathrm{w} - \mathrm{D}_\mathrm{w}}{\mathrm{T}_\mathrm{w} - \mathrm{D}_\mathrm{w}} \times 100 \tag{5}$$

where F_w = fresh weight of leaf sample; D_w = dry weight of leaf sample; and T_w = turgid weight of leaf sample.

Chlorophyll content: The leaf chlorophyll contents were measured by taking absorbance readings at 480, 510, 645, and 663 nm wavelengths against a blank one with only 80% acetone in a Systronics-105 spectrophotometer. The chlorophyll a and b, total chlorophyll, and carotenoid were estimated with the following formula given by [42], all expressed in mg g^{-1} of fresh leaf weight:

$$\text{Chlorophyll a} = (12.7 \times A_{663}) - (2.69 \times A_{645}) \times V/W \times 1000 \tag{6}$$

$$\text{Chlorophyll b} = (22.9 \times A_{665}) - (4.68 \times A_{663}) \times V/W \times 1000 \tag{7}$$

$$\text{Total chlorophyll} = (20.2 \times A_{645}) + (8.02 \times A_{663}) \times V/W \times 1000 \tag{8}$$

$$\text{Carotenoid} = (7.6 \times A_{480}) - (1.49 \times A_{510}) \times V/W \times 1000 \tag{9}$$

where V = extract volume (ml); W = fresh weight of leaf tissue (g), and A = absorbance.

Proline: Free proline contents in the leaves were determined as per the method of Bates et al. [43]. The leaf sample of 100 mg was homogenized in 10 mL of sulpho-salicylic acid (3%) by using mortar and pestle. It was centrifuged at 6000 rpm for 10 min and the supernatant was collected. The 2.0 mL of the extract was taken in the test tube with 2 mL each of glacial acetic acid and ninhydrin reagent was added. The reaction mixture was boiled in water bath at 100 °C for 30 min until a brick red color developed. After cooling to the reaction mixture, 5 mL of toluene was added and then transferred to separating funnel and the absorbance read at 520 nm using a spectrophotometer against toluene as blank.

Nitrate reductase activity: Nitrate reductase activity in freshly harvested leaf tissues (µmol g^{-1} fresh leaf weight $hour^{-1}$) was estimated by following the method of Singh and Nair [44]. Then 250 mg of fresh leaf tissue was taken and rinsed in cold distilled water thoroughly. The sample was cut in small pieces with blade in ice bath. The pieces were suspended properly in 5 mL of medium taken in a clean test tube. The medium contained potassium phosphate buffer (pH 7.6), 0.1 M KNO_3, n-propanol, chloramphenicol, and distilled water. The tubes were kept in the dark after sealing at 25 °C for 25 min. Then 2 mL of aliquots were taken from both the sample and blank tube separately. One mL of 1% sulphanilamide was added in each tube and mix properly. After that, 1 mL of 0.02% NEDA was added in each case with thorough mixing. After 10 min, the contents were diluted by the addition of 1 mL of distilled water. Finally, the pinkish-brown was read against the blank at 540 nm wavelength in a UV-Vis spectrophotometer. The standard curve was prepared to take different aliquots of the working standard of KNO_2 (100 µM).

$$V = \frac{(T - B)}{W \times T} \tag{10}$$

where, V = µmol of NO^{-2} (µmol g^{-1} fresh weight $hour^{-1}$); T = concentration of treatment absorbance; B = concentration of blank absorbance; W = weight of leaf sample (g); and T = incubation period (hour).

Cell membrane stability: Cell membrane stability was measured according to Deshmukh et al. [45]. Fresh leaf samples of 0.1 g from each plot were collected, thoroughly washed in clean water, and the leaf leachates were kept for 30 min at room temperature. Subsequently, the electrical conductivity was taken by using a conductivity bridge (C_1). Next to this, these samples were placed in a boiling distilled water bath (100 °C) for 10 min and again their electrical conductivity was recorded (C_2). Cell membrane stability was calculated with the following Equation (11):

$$\text{Cell membrane stability } (\%) = \left[1 - \left(\frac{C_1}{C_2}\right)\right] \times 100 \tag{11}$$

where, C_1 = initial electrical conductivity of leaf leachates and C_2 = final electrical conductivity of leaf leachates.

2.6. Statistical Analysis

The data were statistically analyzed by applying the technique of analysis of variance (ANOVA) for split–split split-design [46]. Pooled analysis was exercised in case of alike data of both years. Treatment means were compared by employing the F-test. The significant differences between the treatments were compared by critical difference at 5% level of significance. Tukey's post hoc test was performed to compare the differences between mean values.

3. Results

3.1. Thermal Regime and Rainfall Pattern during Black Gram Growth

Weekly distribution of temperature (maximum and minimum), rainfall, and relative humidity evidently varied in different phelological stages during the growing season of black gram under different sowing dates for the two consecutive years (Table 1 and Figures 1 and 2). The temperature ranged from 21.0–34.0 and 22.3–37.2 °C at the time of flowering to pod formation (35–50 days) during 2020 and from 22.7–37.8 and 23.3–38.4 °C during 2021 for the March first week sown and March third week sown crops, respectively. Generally, the spring–summer season in Eastern India is characterized by unpredictable rainfall patterns. The March first week and third week sown crop received the corresponding rainfall of 6.1 and 0.2 cm from vegetative to the flowering period (15–35 DAS) and the afterwards negligible amount was recorded upto pod development in the spring–summer season of 2020. On the other hand, this very season in 2021 experienced a little rainfall in total starting from sowing to pod development of black gram. No particular pattern was found regarding relative humidity (RH) during both the years irrespective of the sowing dates. It ranged from 50.2–91.5% and 47.1–90.8% respectively for March first week and third week sown crop during 2020. In the next year, these respective crops faced 34.5–89.2% and 27.4–86.4% RH. Though the maximum values ranged always above 85%, the lower minimum values (always below 60%) brought down the daily mean relative humidity throughout the crop growth period. The second year under experimentation experienced comparatively lower mean RH values with respect to the first year in all cases. On the other hand, the later sown crop always faced lower values of RH compared to the normal sown crop.

3.2. Effect of Date of Sowing, Soil Application, and Foliar Spray of Nutrients on Phenology

Phenophase duration of black gram over two years indicated a decline in overall crop duration with deviation in sowing from March first week to third week (Table 3).

The crop sown on March first week completed its life cycle in 82 and 81.8 days in 2020 and 2021, respectively. On the other hand, the March third week sown crop took 78.2 and 78.8 days to get matured in the respective year. However, no uniform variation was observed as such among the different phenophases. The March first week sown black gram took mean durations of 6.5, 11.7, 33.6, and 41.1 days in the first year and 5.8, 11.2, 34.2, and 41.1 in the next year from sowing to emergence, branching, flowering, and pod initiation, respectively, whereas the delayed sown crop finished those following stages of growth in 6.9, 12.5, 32.7, and 40.2 days in 2020 and 6.6, 12.5, 33.3, and 40.2 in 2021, respectively. Faster crop emergence of black gram seedlings was registered irrespective of dates of sowing with soil application of Co. Compared to control, soil application of Co and foliar spray of K+B separately extended the requirements of days to maturity as a whole and found out to be statistically significant over this corresponding treatments to be compared (Table 4). Notably, the factors interacted significantly in the maturity stage; however, the interactions in the rest of the stages were non-significant in some of the cases.

Table 3. Phenophase duration of black gram as influenced by sowing dates and nutrients application.

Treatment	Days to Emergence		Days to Branching		Days to Flowering		Days to Pod Initiation		Days to Maturity	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
Date of Sowing (D)										
D_1	6.5 ± 0.12 b	5.8 ± 0.03 b	11.7 ± 0.11 b	11.2 ± 0.11 b	33.6 ± 0.13 b	34.2 ± 0.06 b	41.1 ± 0.03 b	41.1 ± 0.06 b	82.0 ± 0.03 b	81.8 ± 0.17 b
D_2	6.9 ± 0.06 a	6.6 ± 0.03 a	12.5 ± 0.01 a	12.5 ± 0.05 a	32.7 ± 0.11 a	33.3 ± 0.05 a	40.2 ± 0.07 a	40.2 ± 0.03 a	78.2 ± 0.04 a	78.8 ± 0.15 a
Soil Application of Cobalt (S)										
S_1	7.9 ± 0.10 a	7.2 ± 0.03 a	14.3 ± 0.10 a	13.8 ± 0.11 a	31.7 ± 0.27 a	32.5 ± 0.09 a	39.0 ± 0.06 a	39.0 ± 0.03 a	78.5 ± 0.15 a	77.8 ± 0.12 a
S_2	6.0 ± 0.06 b	6.1 ± 0.09 b	10.8 ± 0.09 b	11.6 ± 0.12 b	33.7 ± 0.30 b	34.1 ± 0.12 b	41.4 ± 0.10 b	41.4 ± 0.09 b	81.7 ± 0.13 b	81.6 ± 0.19 b
Foliar Spray (F)										
F_1	7.0 ± 0.14 a	6.9 ± 0.08 a	12.3 ± 0.012 b	12.4 ± 0.14 b	32.9 ± 0.05 b	33.5 ± 0.05 b	38.6 ± 0.30 d	38.6 ± 0.14 d	78.1 ± 0.17 e	75.8 ± 0.00 e
F_2	7.1 ± 0.08 a	6.3 ± 0.14 a	13.0 ± 0.14 a	12.3 ± 0.09 b	32.5 ± 0.14 b	32.8 ± 0.14 b	39.5 ± 0.14 c	39.5 ± 0.14 c	79.4 ± 0.08 d	78.5 ± 0.14 d
F_3	6.8 ± 0.17 a	6.9 ± 0.08 a	12.5 ± 0.08 a	12.6 ± 0.14 a	32.7 ± 0.17 a	33.3 ± 0.14 a	40.2 ± 0.30 b	40.2 ± 0.17 b	80.4 ± 0.22 c	79.8 ± 0.25 c
F_4	6.8 ± 0.17 a	6.3 ± 0.17 a	12.3 ± 0.09 b	12.9 ± 0.07 a	32.8 ± 0.17 a	33.8 ± 0.22 a	41.0 ± 0.14 a	41.0 ± 0.00 a	81.1 ± 0.22 b	81.8 ± 0.25 b
F_5	7.0 ± 0.00 a	6.8 ± 0.17 a	12.7 ± 0.12 a	12.5 ± 0.10 b	33.3 ± 0.17 a	34.6 ± 0.17 a	41.8 ± 0.14 a	41.8 ± 0.00 a	81.8 ± 0.08 a	83.6 ± 0.17 a

Values are means ± SEM (n = 3). Different letters designate significant differences between means. D_1: March first week and D_2: March third week; S_1: RDF (20:40:40 kg N: P_2O_2: K_2O ha^{-1}) and S_2: RDF + soil application of Co at 4 kg ha^{-1} $Co(NO_3)_2$; F_1: No spray, F2: Foliar spray of tap water, F_3: Foliar spray of K at 1.25% (Muriate of Potash); F_4: Foliar spray of B at 0.2% (Borax) and F_5: Foliar spray of K at 1.25% + B at 0.2%.

Table 4. Statistical significance and interaction effects of date of sowing, soil application of Co, and foliar spray of K and B on the phenology of black gram during the spring–summer season.

Phenological Parameters	Seasons	Statistical Significance	Factor Wise Effect			Interaction Effect of All Treatments			
			D	S	F	D × S	D × F	S × F	D × S × F
Days to emergence	2020	SEm(±)	0.09	0.08	0.17	0.15	0.23	0.23	0.33
		LSD	0.58	0.32	NS	NS	NS	NS	NS
	2021	SEm(±)	0.08	0.07	0.16	0.22	0.09	0.22	0.35
		LSD	0.52	0.26	NS	0.64	NS	NS	NS
Days to branching	2020	SEm(±)	0.08	0.34	0.29	0.48	0.42	0.42	0.59
		LSD	0.52	1.34	NS	NS	NS	NS	NS
	2021	SEm(±)	0.17	0.14	0.24	0.21	0.33	0.33	0.47
		LSD	1.05	0.57	NS	NS	NS	NS	NS
Days to flowering	2020	SEm(±)	0.06	0.32	0.21	0.23	0.37	0.37	0.52
		LSD	0.38	1.26	0.60	0.67	NS	NS	NS
	2021	SEm(±)	0.04	0.12	0.13	0.18	0.02	0.18	0.26
		LSD	0.25	0.47	0.38	0.05	NS	NS	NS
Days to pod initiation	2020	SEm(±)	0.15	0.06	0.17	0.08	0.24	0.24	0.33
		LSD	0.95	0.22	0.48	NS	NS	0.68	NS
	2021	SEm(±)	0.05	0.10	0.13	0.16	0.18	0.18	0.26
		LSD	0.29	0.41	0.37	NS	NS	NS	NS
Days to maturity	2020	SEm(±)	0.07	0.14	0.17	0.20	0.25	0.25	0.35
		LSD	0.44	0.55	0.50	0.43	0.71	0.72	1.01
	2021	SEm(±)	0.02	0.06	0.13	0.09	0.08	0.18	0.25
		LSD	0.14	0.24	0.36	0.34	0.51	0.51	0.73

NS: Non-significant; D, Date of sowing, S, Soil application, and F, Foliar spray; SEm (±): Standard Error of mean; LSD: Least Significant Difference.

3.3. Effect of Date of Sowing, Soil Application, and Foliar Spray of Nutrients on Growth Traits

Initial growth stages of black gram attained progressive expansion in LAI, total dry matter, CGR, and NAR, irrespective of sowing dates and years (Figure 3). However, the CGR and NAR followed a gradually decreasing trend from flowering towards pod developmental stages during both years. The March first week sown crop accumulated greater total dry matter compared to the later sown one in both years. NAR was comparatively higher in the first year (Figure 3A_1,A_2) at the interval of vegetative to flowering stages than the second year (Figure 3B_1,B_2) under both the dates of sowing.

Maximum LAI of 3.18 and 3.12 were observed for the March first week sown crop and those of 3.12 and 3.08 for the later sown one during 2020 and 2021, respectively. Significant variations during flowering to pod development in all the cases were observed with Co application and foliar nutrition (Table 5). Interestingly, the interactions among all the three factors were statistically significant in all cases of the respective years (Table 6).

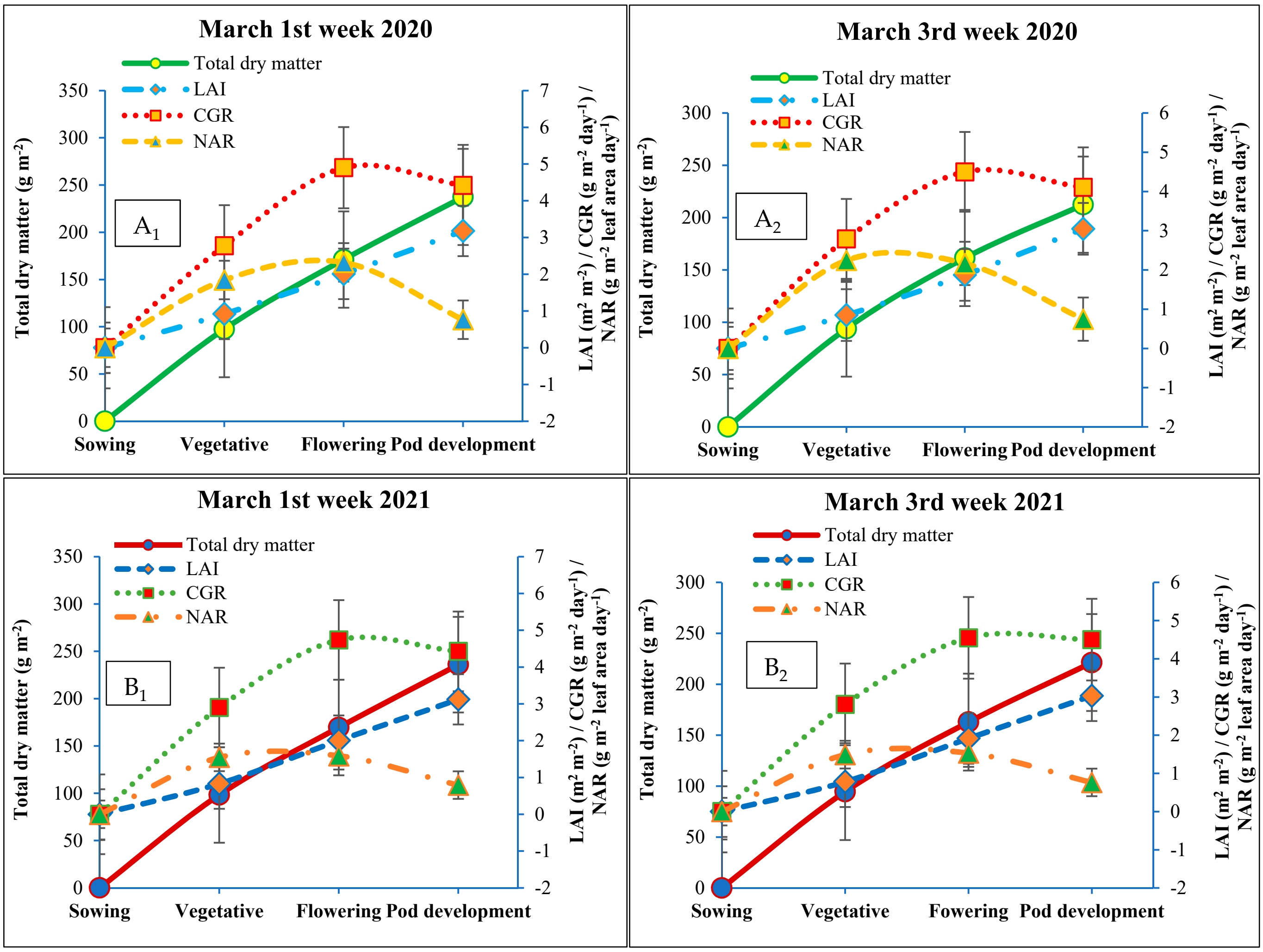

Figure 3. Growth characteristics at different phenophases under March first week sowing during (**A_1**) 2020 and (**A_2**) 2021 crop seasons and under March third week sowing during (**B_1**) 2020 and (**B_2**) 2021 crop seasons.

Table 5. Growth parameters of black gram influenced by date of sowing and nutrients application.

Treatment	Total Dry Matter (g m^{-2})		LAI (m^2 m^{-2})		CGR (g m^{-2} day^{-1})		NAR (g m^{-2} Leaf Area day^{-1})	
	2020	2021	2020	2021	2020	2021	2020	2021
Date of Sowing (D)								
D_1	237.3 ± 0.41 a	235.9 ± 0.62 a	3.18 ± 0.02 a	3.12 ± 0.01 a	4.42 ± 0.06 a	4.43 ± 0.10 a	0.76 ± 0.01 a	0.79 ± 0.01 a
D_2	225.5 ± 0.62 b	228.9 ± 0.39 b	3.12 ± 0.00 b	3.08 ± 0.01 b	4.27 ± 0.06 b	4.46 ± 0.07 a	0.75 ± 0.01 a	0.78 ± 0.01 a
Soil Application of Cobalt (S)								
S_1	221.2 ± 1.25 b	225.1 ± 0.66 b	3.04 ± 0.01 b	2.98 ± 0.02 b	4.12 ± 0.03 b	4.43 ± 0.06 a	0.74 ± 0.01 a	0.76 ± 0.02 a
S_2	228.5 ± 0.29 a	232.0 ± 0.53 a	3.19 ± 0.01 a	3.18 ± 0.01 a	4.40 ± 0.011 a	4.50 ± 0.07 a	0.77 ± 0.01 a	0.80 ± 0.01 a
Foliar Spray (F)								
F_1	203.5 ± 0.30 e	212.1 ± 0.72 e	2.94 ± 0.01 e	2.91 ± 0.01 d	3.71 ± 0.06 e	4.15 ± 0.12 d	0.67 ± 0.02 b	0.72 ± 0.01 b
F_2	214.0 ± 1.46 d	222.1 ± 0.30 d	3.03 ± 0.02 d	3.00 ± 0.01 cd	4.01 ± 0.09 c	4.38 ± 0.08 c	0.71 ± 0.01 ab	0.76 ± 0.02 ab
F_3	225.4 ± 1.63 c	229.7 ± 0.90 c	3.13 ± 0.01 c	3.09 ± 0.01 c	4.29 ± 0.13 c	4.49 ± 0.12 b	0.76 ± 0.02 a	0.78 ± 0.02 a
F_4	234.8 ± 0.28 b	234.8 ± 1.39 b	3.20 ± 0.01 b	3.17 ± 0.01 b	4.58 ± 0.07 b	4.52 ± 0.26 b	0.80 ± 0.02 a	0.80 ± 0.01 a
F_5	246.6 ± 0.52 a	244.3 ± 0.87 a	3.28 ± 0.01 a	3.22 ± 0.02 a	4.72 ± 0.05 a	4.78 ± 0.13 a	0.83 ± 0.01 a	0.82 ± 0.01 a

Values are means ± SEM (n = 3). Different letters designate significant differences between means. D_1: March first week and D_2: March third week; S_1: RDF (20:40:40 kg N: P_2O_2: K_2O ha^{-1}) and S_2: RDF + soil application of Co at 4 kg ha^{-1} $Co(NO_3)_2$; F_1: No spray, F2: Foliar spray of tap water, F_3: Foliar spray of K at 1.25% (Muriate of Potash); F_4: Foliar spray of B at 0.2% (Borax); and F_5: Foliar spray of K at 1.25% + B at 0.2%.

Table 6. Statistical significance and interaction effects of date of sowing, soil application of Co and foliar spray of K and B on the growth traits of black gram during spring–summer season.

Growth Parameters	Seasons	Statistical Significance	Factor Wise Effect			Interaction Effect of All Treatments			
			D	S	F	D × S	D × F	S × F	D × S × F
Total dry matter	2020	SEm(±)	0.34	0.72	0.89	1.03	1.27	1.27	1.79
		LSD	2.08	2.83	2.58	3.03	3.65	3.65	5.16
	2021	SEm(±)	0.42	0.58	0.89	0.82	1.26	1.26	1.78
		LSD	2.62	2.26	2.56	2.60	3.62	3.60	5.12
LAI	2020	SEm(±)	0.01	0.01	0.01	0.01	0.01	0.01	0.02
		LSD	0.07	0.02	0.02	0.03	NS	0.02	0.06
	2021	SEm(±)	0.01	0.01	0.01	0.01	0.01	0.01	0.02
		LSD	0.02	0.02	0.02	0.02	NS	0.03	0.04
CGR	2020	SEm(±)	0.04	0.03	0.07	0.04	0.11	0.11	0.15
		LSD	0.10	0.08	0.22	0.17	0.30	0.30	0.31
	2021	SEm(±)	0.01	0.03	0.05	0.04	0.07	0.07	0.10
		LSD	0.09	0.10	0.14	0.14	0.20	0.20	0.28
NAR	2020	SEm(±)	0.01	0.01	0.01	0.01	0.01	0.01	0.02
		LSD	0.02	0.02	0.02	NS	0.03	NS	0.05
	2021	SEm(±)	0.01	0.01	0.01	0.01	0.01	0.01	0.02
		LSD	0.01	0.02	0.03	NS	0.02	NS	0.04

NS: Non-significant; D, Date of sowing, S, Soil application, and F, Foliar spray; SEm (±): Standard Error of mean; LSD: Least Significant Difference.

3.4. Effect of Date of Sowing, Soil Application, and Foliar Spray of Nutrients on Relative Leaf Water Content

Significant differences were recorded in RLWC of black gram over the dates of sowing, soil, and foliar application of nutrients (Tables 7 and 8). The results of the RLWC of two years are presented in Table 7. In all the time intervals, the March first week sown crop contained a higher amount of relative water in leaf tissues in contrast to that of the delayed sown one. Among the soil-applied treatments, Co application resulted in significant higher RLWC compared to control ranging from 98.4% and 98.5% at vegetative, 90.7% and 90.0% at flowering, to 88.0% and 88.2% at pod developmental stage of black gram in 2020 and 2021, respectively (Tables 7 and 8). Regarding the foliar-treated plots, 1.25% K surpassed the 0.2% B spray in this regard. However, the combined spray of K+B turned out to be the best measure to maintain higher RLWC of 89.8% and 89.5% at flowering and 86.8 and 88.2% at pod filling stages in the respective years.

Table 7. Relative leaf water content at different growth stages.

Treatment	Relative Leaf Water Content (%)							
	15 DAS		30 DAS		45 DAS		60 DAS	
	2020	2021	2020	2021	2020	2021	2020	2021
Date of Sowing (D)								
D_1	97.4 ± 0.1 a	97.9 ± 0.1 a	94.3 ± 0.4 a	94.1 ± 0.1 a	89.7 ± 0.1 a	89.7 ± 0.1 a	86.9 ± 0.0 a	87.8 ± 0.1 a
D_2	96.9 ± 0.1 b	97.5 ± 0.1 a	93.6 ± 0.2 b	93.4 ± 0.1b	89.0 ± 0.1 a	89.0 ± 0.0 a	86.0 ± 0.1 a	87.5 ± 0.1 a
Soil Application of Cobalt (S)								
S_1	95.4 ± 0.1 b	96.4 ± 0.1 b	92.1 ± 0.2 b	92.5 ± 0.3 b	87.4 ± 0.1 b	87.8 ± 0.1 b	83.9 ± 0.1 b	86.7 ± 0.1 b
S_2	98.4 ± 0.1 a	98.5 ± 0.1 a	94.9 ± 0.3 a	94.2 ± 0.2 a	90.7 ± 0.1 a	90.0 ± 0.1 a	88.0 ± 0.2 a	88.2 ± 0.1 a
Foliar Spray (F)								
F_1	94.2 ± 0.1 b	95.6 ± 0.1 b	93.4 ± 0.4 a	93.1 ± 0.3 a	88.3 ± 0.1 b	88.3 ± 0.1 a	85.2 ± 0.1 b	86.6 ± 0.1 c
F_2	96.6 ± 0.1 a	97.3 ± 0.1 a	93.6 ± 0.3 a	93.4 ± 0.4 a	88.7 ± 0.1 ab	88.6 ± 0.1 a	85.6 ± 0.2 b	87.1 ± 0.2 b
F_3	96.8 ± 0.1 a	97.6 ± 0.1 a	93.5 ± 0.3 a	93.5 ± 0.1 a	89.3 ± 0.1 a	89.1 ± 0.2 a	86.3 ± 0.1 a	87.8 ± 0.1 b
F_4	97.1 ± 0.1 a	97.6 ± 0.1 a	93.7 ± 0.4 a	93.4 ± 0.1 a	89.0 ± 0.1 a	89.1 ± 0.1 a	85.9 ± 0.1 b	87.5 ± 0.1 b
F_5	96.9 ± 0.1 a	97.6 ± 0.0 a	93.5 ± 0.1 a	93.3 ± 0.1 a	89.8 ± 0.1 a	89.5 ± 0.1 a	86.8 ± 0.1 a	88.2 ± 0.1 a

Values are means ± SEM (n = 3). Different letters designate significant differences between means. D_1: March first week and D_2: March third week; S_1: RDF (20:40:40 kg N: P_2O_2: K_2O ha^{-1}) and S_2: RDF + soil application of Co at 4 kg ha^{-1} $Co(NO_3)_2$; F_1: No spray, F2: Foliar spray of tap water, F_3: Foliar spray of K at 1.25% (Muriate of Potash); F_4: Foliar spray of B at 0.2% (Borax) and F_5: Foliar spray of K at 1.25% + B at 0.2%.

Table 8. Statistical significance and interaction effects of date of sowing, soil application of Co and foliar spray of K and B on the relative leaf water content of black gram during the spring–summer season.

RLWC at Time Interval	Seasons	Statistical Significance	Factor Wise Effect			Interaction Effect of All Treatments			
			D	S	F	D × S	D × F	S × F	D × S × F
15 DAS	2020	SEm(±)	0.09	0.07	0.18	0.10	0.25	0.25	0.36
		LSD	0.53	0.29	NS	NS	NS	NS	NS
	2021	SEm(±)	0.08	0.25	0.15	0.09	0.21	0.21	0.30
		LSD	0.49	0.06	NS	NS	NS	NS	NS
30 DAS	2020	SEm(±)	0.14	0.13	0.18	0.18	0.26	0.26	0.37
		LSD	0.84	0.51	NS	0.72	NS	NS	NS
	2021	SEm(±)	0.10	0.11	0.15	0.16	0.21	0.21	0.30
		LSD	0.64	0.45	NS	NS	NS	NS	NS
45 DAS	2020	SEm(±)	0.22	0.15	0.25	0.22	0.35	0.35	0.50
		LSD	0.53	0.41	0.72	0.85	1.01	1.01	1.44
	2021	SEm(±)	0.15	0.09	0.19	0.14	0.27	0.27	0.38
		LSD	0.92	0.37	0.54	0.53	0.77	0.77	1.09
60 DAS	2020	SEm(±)	0.26	0.18	0.30	0.25	0.42	0.42	0.59
		LSD	0.48	0.49	0.85	0.99	1.21	1.21	1.71
	2021	SEm(±)	0.15	0.09	0.19	0.14	0.27	0.27	0.38
		LSD	0.92	0.37	0.53	0.54	0.77	0.77	1.09

RLWC-Relative leaf water content; NS: Non-significant; D, Date of sowing, S, Soil application, and F, Foliar spray; SEm (±): Standard Error of mean; LSD: Least Significant Difference.

3.5. Effect of Date of Sowing, Soil Application, and Foliar Spray of Nutrients on Leaf Chlorophyll and Carotenoid Contents

Superior values of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid were observed in March first week sowing (1.08, 0.28, 1.36, and 0.34 mg g^{-1} of fresh weight) compared to March first week sown crop (0.97, 0.26, 1.23, and 0.31) in the year 2020 (Figure 4A). A similar trend was followed in the next year (Figure 4B) too. However, the overall range of all pigments exhibited lower values in 2021 than in 2020. Soil application of Co, as well as foliar nutrition, had a significant constructive role in terms of improving the leaf chlorophyll content. During the respective years, incorporation of Co recorded chlorophyll-a of 1.05 and 0.97, chlorophyll b of 0.25 and 0.24, total chlorophyll of 1.24–1.12, and finally carotenoid of 0.34–0.29 all expressed in terms of mg g^{-1} of fresh weight of black gram leaf irrespective of sowing date. Regarding the foliar spray treatments, foliar K and B either of single or in combination attained higher pigment concentrations compared to control during both years. However, foliar spray with K+B gave rise to significantly greater values of their corresponding pigments over others followed by a single K spray in each of the years.

3.6. Effect of Date of Sowing, Soil Application, and Foliar Spray of Nutrients on Proline Profile

Proline content of spring–summer black gram varied significantly when sown on different dates under different soil and foliar nutrition regardless of the years under study (Tables 9 and 10). The March first week sown crop recorded higher proline accumulation compared to that of March third week sown one (4.59 vs. 4.41 and 4.30 vs. 4.21 mg g^{-1} leaf fresh weight) during the respective years. In comparison with control, application of Co in soil also resulted in a significant build-up of proline profile in leaf tissues (4.42 vs. 4.77 and 4.42 vs. 4.46 mg g^{-1} leaf fresh weight), respectively, in 2020 and 2021, irrespective of sowing dates. However, proline accumulation ranged between 4.38 to 4.80 and 4.16 to 4.53 mg g^{-1} leaf fresh weight among the foliar sprayed treatments, combined with K+B being the best one in the consecutive years, respectively.

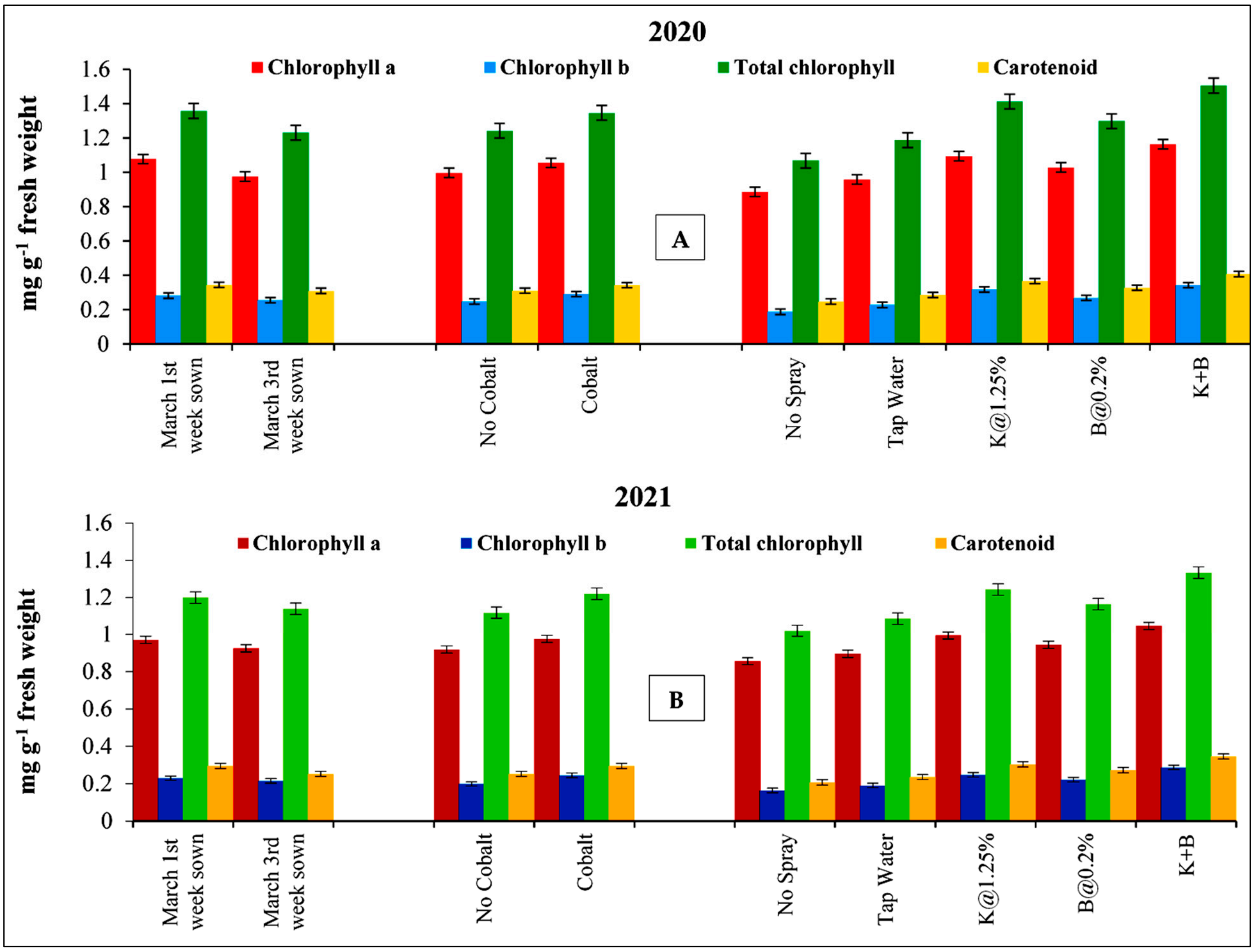

Figure 4. Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents of black gram leaves under different dates of sowing and different nutrient applications during crop season: (**A**) 2020 and (**B**) 2021 (error bars are representing the standard error of the mean).

Table 9. Proline content, nitrate reductase content, and cell membrane stability of black gram under different dates of sowing and different nutrient applications.

Treatment	Proline Content (mg g^{-1} Leaf Fresh Weight)		Nitrate Reductase Content (μmol g^{-1} Leaf Fresh Weight $hour^{-1}$)		Cell Membrane Stability (%)	
	2020	2021	2020	2021	2020	2021
			Date of Sowing (D)			
D_1	4.59 ± 0.01 a	4.30 ± 0.2 a	1.76 ± 0.02 a	1.97 ± 0.02 a	55.67 ± 0.72 a	52.77 ± 0.23 a
D_2	4.41 ± 0.01 b	4.21 ± 0.03 b	1.60 ± 0.02 b	1.83 ± 0.02 b	51.53 ± 0.57 b	50.59 ± 0.31 b
			Soil Application of Cobalt (S)			
S_1	4.42 ± 0.01 b	4.42 ± 0.01 a	1.48 ± 0.01 b	1.69 ± 0.02 b	46.98 ± 0.41 b	48.19 ± 0.41 b
S_2	4.77 ± 0.01 a	4.46 ± 0.02 a	1.71 ± 0.03 a	1.96 ± 0.02 a	55.67 ± 0.72 a	52.77 ± 0.23 a
			Foliar Spray (F)			
F_1	4.38 ± 0.01 d	4.16 ± 0.03 e	1.41 ± 0.02 d	1.67 ± 0.03 d	45.08 ± 0.68 e	46.69 ± 0.29 e
F_2	4.49 ± 0.01 cd	4.25 ± 0.03 d	1.51 ± 0.03 c	1.75 ± 0.03 c	47.81 ± 0.56 d	48.49 ± 0.59 d
F_3	4.71 ± 0.01 b	4.43 ± 0.03 b	1.68 ± 0.03 b	1.89 ± 0.02 b	51.75 ± 0.75 c	50.38 ± 0.36 c
F_4	4.59 ± 0.01 c	4.34 ± 0.01 c	1.61 ± 0.02 b	1.83 ± 0.0 b	54.66 ± 0.29 b	52.53 ± 0.13 b
F_5	4.80 ± 0.01 a	4.53 ± 0.01 a	1.77 ± 0.01 a	1.98 ± 0.03 a	57.33 ± 0.74 a	54.31 ± 0.34 a

Values are means ± SEM (n = 3). Different letters designate significant differences between means. D_1: March first week and D_2: March third week; S_1: RDF (20:40:40 kg N: P_2O_2: K_2O ha^{-1}) and S_2: RDF + soil application of Co at 4 kg ha^{-1} $Co(NO_3)_2$; F_1: No spray, F2: Foliar spray of tap water, F_3: Foliar spray of K at 1.25% (Muriate of Potash); F_4: Foliar spray of B at 0.2% (Borax) and F_5: Foliar spray of K at 1.25% + B at 0.2%.

Table 10. Statistical significance and interaction effects of date of sowing, soil application of Co and foliar spray of K and B on the proline content, nitrate reductase content, and cell membrane stability of black gram during the spring–summer season.

Parameters	Seasons	Statistical Significance	Factor Wise Effect			Interaction Effect of All Treatments			
			D	S	F	D × S	D × F	S × F	D × S × F
Proline content	2020	SEm(±)	0.01	0.01	0.01	0.01	0.02	0.02	0.02
		LSD	0.03	0.02	0.03	0.03	0.04	0.04	0.04
	2021	SEm(±)	0.01	0.01	0.01	0.01	0.01	0.01	0.01
		LSD	0.03	0.02	0.02	0.03	0.02	NS	0.02
Nitrate reductase content	2020	SEm(±)	0.01	0.01	0.01	0.01	0.01	0.01	0.01
		LSD	0.05	0.02	0.02	NS	0.02	0.02	NS
	2021	SEm(±)	0.01	0.01	0.01	0.01	0.01	0.01	0.01
		LSD	0.04	0.02	0.02	NS	0.03	0.03	NS
Cell membrane stability	2020	SEm(±)	0.13	0.17	0.19	0.24	0.27	0.27	0.39
		LSD	0.79	0.67	0.56	0.95	0.79	0.75	1.12
	2021	SEm(±)	0.09	0.13	0.15	0.19	0.22	0.22	0.31
		LSD	0.53	0.51	0.44	0.61	0.62	0.63	0.64

NS: Non-significant; D, Date of sowing, S, Soil application, and F, Foliar spray; SEm (±): Standard Error of mean; LSD: Least Significant Difference.

3.7. Effect of Date of Sowing, Soil Application, and Foliar Spray of Nutrients on Nitrate Reductase Activity

Black gram sown in spring–summer attained a significantly higher amount of leaf nitrate reductase (NR) content when sown on March first week (1.76 and 1.97 µmol g^{-1} leaf fresh weight $hour^{-1}$) over its delayed sowing (1.60 and 1.83 µmol g^{-1} leaf fresh weight $hour^{-1}$) in 2020 and 2021 respectively (Tables 9 and 10). In addition, nutrient application in terms of Co (soil-applied) and K+B (foliar applied) separately recorded significant NR content over control during both years.

3.8. Effect of Date of Sowing, Soil Application, and Foliar Spray of Nutrients on Cell Membrane Stability

Cell membrane stability (CMS) with respect to spring–summer black gram is depicted in Table 9. Around 8% and 4% deviations in CMS were noted with the crop sown on March third week which was subjected to more moisture and heat stress as compared to the crop of March first week in the consecutive years. Plants grown with Co application in 2020 and 2021, respectively, showed CMS to the tune of 55.67% and 52.77%, statistically significant over control. The foliar treatments also differed significantly among themselves (Table 10), attaining the highest CMS with combined K+B spray treatment (57.33% and 54.31%) during the respective years.

3.9. Black gram Yield as Influenced by Date of Sowing, Soil Application, and Foliar Spray of Nutrients

Variation in seed yield of black gram grown in spring–summer depicted the effectiveness of the treatments of individual factors of the experiment irrespective of the years. The black gram crop attained a seed yield of 1278.4 and 1225.4 kg ha^{-1} as well as stover yield to the tune of 1667.7and 1464.4 kg ha^{-1}, respectively, in two years when sown in March first week (Table 11).

This sowing date was accompanied by a comparatively lower mean daily temperature during the whole reproductive development period of the crop which enabled it to achieve 18.3% and 13.0% more economic yield than that of the crop sown in March third week (1126.0 and 1152.8 kg ha^{-1}) in 2020 and 2021, respectively. Soil application of Co and foliar spray of K+B separately accounted for significant higher seed yield and stover yield compared to their corresponding control irrespective of sowing dates during both the years. All the interaction effects among the factors were statistically significant regarding both seed and stover yield of blackgram (Table 12). Accordingly, the harvest index of black gram also differed with the sowing dates and nutrient application. Separately, the March first week sowing, Co application, and foliar K+B registered 43.1%, 44.4%, and 44.2% in 2020 and 45.9%, 46.9%, and 47.1% harvest index in 2021, respectively, which were statistically significant over their corresponding treatments to be compared (Tables 11 and 12). However, only the date of sowing and Co application interacted significantly among themselves with respect to harvest index in the respective years.

Table 11. Yield characteristics of black gram under different dates of sowing and different nutrient.

Treatment	Pods per Plant		Seed Yield (kg ha^{-1})		Stover Yield (kg ha^{-1})		Harvest Index (%)	
	2020	2021	2020	2021	2020	2021	2020	2021
Date of Sowing (D)								
D_1	33.3 ± 0.1 a	34.2 ± 0.4 a	1378.4 ± 11.74 a	1325.4 ± 6.45 a	1676.7 ± 19.4 a	1464.4 ± 35.0 a	43.1 ± 0.7 a	45.9 ± 0.5 a
D_2	30.8 ± 0.2 b	30.9 ± 0.8 b	1126.0 ± 7.62 b	1152.8 ± 2.14 b	1525.1 ± 8.4 b	1353.2 ± 22.5 b	42.7 ± 0.4 b	45.3 ± 0.5 b
Soil Application of Cobalt (S)								
S_1	26.9 ± 0.6 b	28.0 ± 0.6 b	1138.7 ± 9.05 b	1052.2 ± 5.78 b	1592.7 ± 10.6 b	1276.5 ± 36.6 b	41.5 ± 0.5 b	44.9 ± 0.5 b
S_2	34.4 ± 0.8 a	33.5 ± 1.0 a	1308.2 ± 6.48 a	1246.1 ± 8.09 a	1652.3 ± 10.6 a	1416.9 ± 9.2 a	44.4 ± 0.3 a	46.9 ± 0.4 a
Foliar Spray (F)								
F_1	20.0 ± 0.6 e	19.5 ± 0.7 e	878.7 ± 10.61 e	838.3 ± 4.64 e	1257.6 ± 30.1 e	1043.3 ± 19.9 e	41.0 ± 0.5 c	44.7 ± 0.2 c
F_2	25.8 ± 0.4 d	24.9 ± 0. 5d	1075.7 ± 6. 54 d	1021.1 ± 5.53 d	1478.1 ± 11.3 d	1187.0 ± 7.7 d	42.0 ± 0.8 b	45.5 ± 0.4 b
F_3	31.3 ± 0.6 c	30.8 ± 0.8 c	1239.4 ± 10.93 c	1161.1 ± 9.53 c	1642.6 ± 26.1 c	1373.3 ± 44.6 c	42.9 ± 0.5 b	45.8 ± 0.9 b
F_4	36.5 ± 0.3 b	36.3 ± 1.2 b	1392.7 ± 4.55 b	1293.0 ± 8.77 b	1801.8 ± 19.9 b	1501.3 ± 11.8 b	43.6 ± 0.2 ab	46.4 ± 0.7 a
F_5	39.8 ± 0.1 a	42.0 ± 0.9 a	1530.6 ± 5.74 a	1432.2 ± 8.78 a	1932.4 ± 53.2 a	1635.8 ± 59.0 a	44.2 ± 0.8 a	47.1 ± 0.6 a

Values are means ± SEM (n = 3). Different letters designate significant differences between means. D_1: March first week and D_2: March third week; S_1: RDF (20:40:40 kg N: P_2O_2: K_2O ha^{-1}) and S_2: RDF + soil application of Co at 4 kg ha^{-1} $Co(NO_3)_2$; F_1: No spray, F2: Foliar spray of tap water, F_3: Foliar spray of K at 1.25% (Muriate of Potash); F_4: Foliar spray of B at 0.2% (Borax) and F_5: Foliar spray of K at 1.25% + B at 0.2%.

Table 12. Statistical significance and interaction effects of date of sowing, soil application of Co and foliar spray of K and B on the yield characteristics of black gram during spring–summer season.

Parameter	Seasons	Statistical Significance	Factor Wise Effect			Interaction Effect of All Treatments			
			D	S	F	D × S	D × F	S × F	D × S × F
Number of pods per plant	2020	SEm(±)	0.18	0.33	0.49	0.47	0.69	0.69	0.98
		LSD	1.10	1.29	1.41	1.83	1.61	1.62	2.12
	2021	SEm(±)	0.45	0.38	0.56	0.53	0.79	0.79	1.13
		LSD	2.80	1.47	1.62	2.08	2.22	2.19	2.54
Seed yield	2020	SEm(±)	8.69	6.42	10.15	9.08	14.36	14.36	20.31
		LSD	53.61	18.43	29.13	26.06	43.02	43.02	56.27
	2021	SEm(±)	9.89	7.01	11.80	9.91	16.69	16.69	23.60
		LSD	61.06	27.37	34.00	23.60	48.09	48.00	71.03
Stover yield	2020	SEm(±)	9.57	4.97	18.02	7.03	25.48	25.48	36.04
		LSD	59.03	19.41	51.93	27.46	73.43	74.36	84.43
	2021	SEm(±)	12.48	11.33	14.07	16.02	19.89	19.89	28.14
		LSD	76.99	44.24	40.54	44.58	52.49	52.49	78.32
Harvest index	2020	SEm(±)	0.28	0.21	0.32	0.29	0.46	0.46	0.65
		LSD	NS	0.80	0.94	1.14	1.32	NS	NS
	2021	SEm(±)	0.22	0.13	0.26	0.18	0.36	0.36	0.51
		LSD	NS	0.50	0.74	0.71	NS	NS	NS

NS: Non-significant; D, Date of sowing, S, Soil application, and F, Foliar spray; SEm (±): Standard Error of mean; LSD: Least Significant Difference.

Variation in the date of sowing also determined the number of pods coming in individual black gram plants regardless of the study years. About 34 and in 2020 and 28 pods in 2021 were observed in the March first week and third week sown crop. Application of Co and combined foliar sprays of K+B also resulted in a significant higher pod set (compared to that of black gram grown without that nutrition. Notably, the seed yield of black gram was detected to have a strong linear correlation with the number of pods per plant during both the years (Figure 5A,B). About 96.59% and 93.98% variations in seed yield could be explained through the fluctuations in pod number per plant in the respective years.

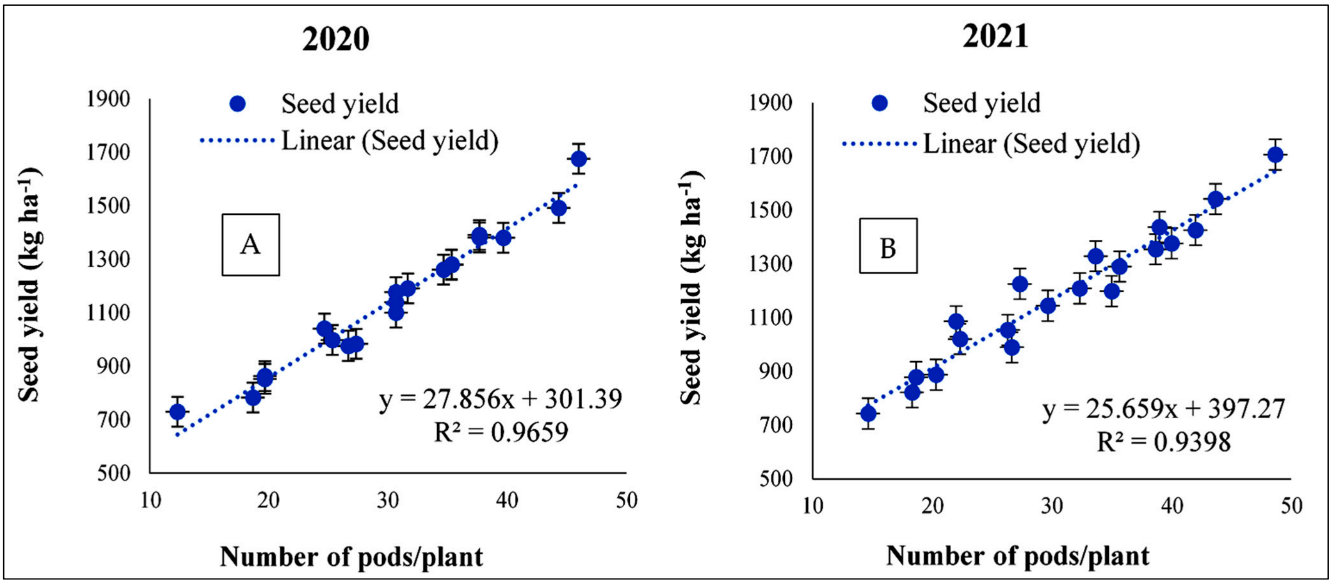

Figure 5. Impact of the number of pods per plant on seed yield of black gram during crop season: (**A**) 2020 and (**B**) 2021 (error bars are representing the standard error of the mean.).

4. Discussion

4.1. Phenology

The onset of every phenophase in any crop is largely governed by the prevailing atmospheric temperature. In fact, alterations in daily maximum and minimum temperatures reduce the time span of phenological stages and retard crop growth processes [47]. The March third week sown black gram experienced a higher mean temperature on the whole as compared to the March first week sown one (Table 1). In succession, the later sown crop was exposed to moisture and heat stress resulting in early flowering and a shorter life cycle [48,49]. Though the variations between growth stages were not standardized, the marked influence of the interval between sowing to flowering on the gross life cycle of black gram was established from this study. The application of Co and foliar K+B might have triggered the production of flowers, extending the maturity [50].

4.2. Growth Traits

From flowering onwards, the crop started facing a persistent rise in atmospheric temperature and a decrease in soil moisture storage due to lack of rainfall and irrigation. As a consequence, the crop might have survived transpiring more but with lesser water uptake [51]. Probably this phenomenon caused the CGR and NAR to decline gradually. This finding was in line with that of Yohan et al. [52] regarding moisture stress-induced reduction in CGR and NAR of black gram during pod initiation to seed filling (40–60 DAS) stage. Similar negative impacts of moisture stress on CGR and NAR were also observed in the case of mungbean [53], cluster bean [54], and pigeon pea [55], respectively. Initial Co application was found to be associated with greater leaf area expansion along with restricted leaf senescence [56], which maintained a progressive increment in LAI even after the reproductive growth set in. Additionally, foliar spray at the flower initiation stage happened to be a fantastic way out to flourish with extended leaf area throughout the reproductive phase of black gram even under stress irrespective of dates of sowing [2]. However, the expansion of leaf area was found to be more pronounced in the case of the

March first week sown crop compared to the later sown one in both years. However, the contradictory decrease in CGR and NAR after pod initiation might be due to the enhanced mutual shading of black gram leaves for improved LAI along with increased defoliation owing to moisture stress [57].

4.3. Relative Leaf Water Content

Relative leaf water content (RLWC) is one of the most relevant and appropriate indicators of the internal water status of a plant in terms of cellular water deficiency providing an instant outlook of several abiotic stresses including heat and moisture stress [58]. A decrease in RLWC with the advancement of phenophases of spring–summer sown black gram could be related to subsequent lower water uptake under emerging moisture stress conditions [59]. The magnitude of moisture deficit was higher in the case of a late sown crop (March third week) than the normal one (March first week). This reduction in RLWC might have led to the loss of turgor in delayed sown crop reducing its cellular expansion and division which accordingly suppressed the regular rate and potential of crop growth. Regarding the impact of Co incorporation in soil, it might have regulated the water balance inside the plant system while bringing down the transpirational rate [24], thus maintaining the level of RLWC at an optimum range. In line with this, the foliar spray technology at the flower initiation stage proved to be immensely efficient in keeping up the optimum cellular moisture level under limited soil moisture as reported by Banerjee et al. [60]. Potassium in combination with boron was observed to substantially modulate the internal water balance of black gram by maintaining proper RLWC (Tables 7 and 8) probably through minimization of its transpirational loss and upgrading the water uptake potential of the root system even under moisture stress [33,61].

4.4. Leaf Chlorophyll and Carotenoid Contents

Leaf chlorophyll content of the plant is one of the fundamental attributing characteristics related to photosynthetic capacity. During both 2020 and 2021, treatments under lesser stress achieved better pigment concentrations in either of the sowing dates. However, the date of sowing of black gram had significant relevance in reducing both heat and moisture stress during spring–summer, which might have markedly influenced the chlorophyll and carotenoid contents leading to the improved photosynthetic ability of the plants. Besides, soil-applied Co and foliar applied K and B turned up to be extremely promising in accelerating the biosynthesis of chlorophyll and in retention of their structural integrity even under deficit soil moisture. These findings were in harmony with those of Pegu et al. [62] and Thakur et al. [19].

4.5. Proline Profile and Cell Membrane Stability

Plants pile up several light-weight metabolites or osmoprotectants including sugars, amino acids, and organic acids after getting exposed to environmental stresses to stabilize the internal balance of osmotic regulation [63]. One such kind of amino acid is proline, actively participating in the protection of sub-cellular structures from stress-induced oxidative damage through reactive oxygen species (ROS) scavenging mechanism [64]. Thus, it alleviates drought stress by way of improving the moisture status of plants at cellular levels [65]. Higher accumulation of proline due to Co, K, and B application suggested proficiency of those nutrients to support the crop in acclimatization under moisture stress during spring–summer regardless of sowing dates.

The stability of cell membranes of plants also has a strong interconnection with the availability of moisture [66]. Sowing of black gram in the March first week in both 2020 and 2021 corresponded to moderate mean daily temperature during the reproductive phase, implying lower evapotranspiration and better retention of plant-available soil moisture in the root zone. This fact accordingly justified the departure in CMS in delayed sown black gram. Intensification in membrane stability has been reported to decrease the permeability of cell membrane by arresting leakage of electrolytic compounds across the membrane from the cell [67]. In this regard, Co, K, and B in terms of their respective application techniques remarkably brought down the moisture stress throughout the growing period of black gram in spring–summer, thus sustaining membrane stability.

4.6. Nitrate Reductase (NR) Activity

Technically, NR is the major responsible enzyme catalyzing the nitrate reduction essential for nitrogen assimilation in legumes with special reference to black gram [68]. Stress conditions regarding delayed sowing might have posed a negative impact on leaf NR content of black gram irrespective of nutrient application. This observation was in accordance with that of Singh and Jain [69]. Proper nutrient balance for legumes in terms of Co, K, and B also seemed to assist in optimum regulations of physiological and biochemical mechanisms in black gram, thus maintaining considerable NR activity.

4.7. Yield Traits

Sowing time as well as soil and foliar application of nutrients invariably imposed a substantial impact on the overall biological yield of black gram during the spring–summer season. The March first week sown crop had more time available for both the processes of flower to pod conversion and subsequent seed filling and thus achieved superior yield than the later sown one with reduced reproductive span. On the contrary, the March third week sown crop suffered from both heat and moisture stress during its pod development. Basically, the optimum temperature range for black gram growth has been found to be 22–28 °C [16]. Moreover, most of the warm season tropical as well as subtropical crops are exposed to heat stress when the ambient temperature goes beyond 32–35 °C [5,70]. The higher mean daily maximum temperature coupled with lower mean relative humidity that the delayed sown crop experienced from flowering to pod developmental stage, was visibly beyond the optimum range. Hence, the crop had definitely been exposed to heat stress during these two stages which are much critical from the production point of view of black gram. On the other hand, higher temperatures combined with lower relative humidity have a specific role in increasing the evapotranspiration loss from soil as well as crop canopy, which can imply apparent moisture stress at the reproductive stage of this crop. Decline in relative humidity in the air owing to the higher atmospheric temperature might have substantially attributed to intensify the impacts of heat and moisture stress inside the crop [71,72]. Additionally because of stress, the crop faced adverse impacts on its CGR and LAI, consequently acquiring lesser photosynthetic area and biomass production, hampering seed set and yield potential [73]. This finding resembled the observations of Math et al. [22] in black gram and Kataria et al. [29] and Iram et al. [25] in the case of green gram.

Application of Co invariably accounted for black gram growth with special reference to nitrogenase enzyme activity triggering nodulation and nutrients uptake [74]. Foliar nutrition of K+B spray seemed to potentially encourage considerable symbiotic nitrogen fixation, nitrate reductase activity, flowering, and seed development. In addition, these particular nutrient elements might have played a pivotal role in the amelioration of the adversities of the prevailing heat and moisture stress of spring–summer of both experimental years through regulation of water economy, photosynthetic pigments production, maintenance of cell membrane stability and stimulation of enzymatic and non-enzymatic antioxidant defence mechanism of the crop to a greater extent. This result was in closer agreement with those of Rao et al. [75] and Gowthami et al. [76]. Simultaneously, these nutrients might have also helped in profuse branching and leaf production resulting in higher final biomass production [77,78]. Black gram is a prominent indeterminate crop, an extension of flowering and seed filling in response to optimum sowing time and nutrient application in the form of soil application of Co and foliar K+B remarkably contributed to improved photosynthesizing capacity and better source to sink partitioning eventually brought an about the spectacular increase in pod and seed yield [33,34].

5. Conclusions

Heat stress coupled with moisture stress during the reproductive stage of the delayed sown black gram crop (March third week) compelled it to complete the phenophases to some extent earlier than the normal sown one (March first week). The LAI, CGR, and NAR seemed to be outstanding indices for realistic expression of black gram suffering from moisture shortage. Stress-induced reduction in yield owing to restricted photosynthetic activity and nitrogen assimilation were evident from reduced chlorophyll contents and nitrate reductase activity respectively. Soil application of Co @ 4 kg ha^{-1} and exogenous applications of 1.25% K and 0.2% B alleviated the adversities of those abiotic stresses through the accumulation of proline and maintaining cell membrane stability. The present experiment may conclude that appropriate time of sowing along with nutrient application has a great potential to achieve higher yield in blackgram. In addition to normal sowing, soil application of Co in combination with foliar sprays of K and B proved to be immensely effective in producing satisfactory biomass and sustaining optimum seed yield of blackgram crop (variety: Pant U 31) through relief of stress under delayed sown conditions in Eastern India.

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