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Original article

Optimizing nitrogen supply promotes biomass, physiological characteristics and yield components of soybean (*Glycine max* L. *Merr.*)

Muhammad Saleem Kubar [a], Akhtar Hussain Shar [b], Kashif Ali Kubar [c], Nadir Ali Rind [b], Hidayat Ullah [b], Shahmir Ali Kalhoro [c], Chao Wang [a], Meichen Feng [a], Asadullah Gujar [a], Hui Sun [a], Wude Yang [a,*], Hesham El Enshasy [d,e], Marian Brestic [f], Marek Zivcak [f], Peter Ondrisik [g], Bandar S. Aljuaid [h], Ahmed M. El-Shehawi [h], Mohammad Javed Ansari [i,*]

[a] College of Agriculture, Shanxi Agricultural University, Taigu 030801, PR China
[b] Department of Molecular Biology & Genetics, Faculty of Science & Technology, Shaheed Benazir Bhutto University, Shaheed Benazirabad, Sindh, Pakistan
[c] Faculty of Agriculture, Lasbela University of Agriculture, Water and Marine Sciences, Uthal 90150, Baluchistan, Pakistan
[d] Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia (UTM), Skudai, Johor Bahru 81310, Malaysia
[e] City of Scientific Research and Technology Applications, New Burg Al-Arab, Alexandria 21934, Egypt
[f] Department of Plant Physiology, Slovak University of Agriculture, A. Hlinku 2, 94976 Nitra, Slovakia
[g] Department of Environment and Zoology, Slovak University of Agriculture, Nitra, Tr. A. Hlinku 2, 949 01 Nitra, Slovakia
[h] Department of Biotechnology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia
[i] Department of Botany, Hindu College Moradabad (Mahatma Jyotiba Phule Rohilkhand University Bareilly) India

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ABSTRACT

Avoidable or inappropriate nitrogen (N) fertilizer rates harmfully affect the yield production and ecological value. Therefore, the aims of this study were to optimize the rate and timings of N fertilizer to maximize yield components and photosynthetic parameter of soybean. This field experiment consists of five fertilizer N rates: 0, 75, 150, 225 and 300 kg N ha^{-1} arranged in main plots and four N fertilization timings: V_5 (trifoliate leaf), R_2 (full flowering stage) and R_4 (full poding stage), and R_6 (full seeding stage) growth stages organized as subplots. Results revealed that 225 kg N ha^{-1} significantly enhanced grain yield components, total chlorophyll (Chl), photosynthetic rate (P_N), and total dry biomass and N accumulation by 20%, 16%, 28%, 7% and 12% at R_4 stage of soybean. However, stomatal conductance (g_s), leaf area index (LAI), intercellular CO_2 concentration (*Ci*) and transpiration rate (*E*) were increased by 12%, 88%, 10%, 18% at R_6 stage under 225 kg N ha^{-1}. Grain yield was significantly associated with photosynthetic characteristics of soybean. In conclusion, the amount of nitrogen 225 kg ha^{-1} at R_4 and R_6 stages effectively promoted the yield components and photosynthetic characteristics of soybean.

1. Introduction

Soybean (*Glycine max* L. Merr.) is a major leguminous crop that provides nutrition in the form of vitamins, protein and minerals. Soybean has maximum protein of 30–60% and highest oil contents about 10–30% (Hou et al., 2009). Soybean was patented in East Asian countries, but currently it is broadly grown in tropical, subtropical, and temperate climatic areas (Board 2013). It has been reported that about 50% improve in grain yield is because of genetic development, while the other 50% is attributed to sustainable management practices (Tilman et al., 2011, Henchion et al., 2017, Magrini et al., 2018). The higher percentage of nitrogen was distributed in seeds at harvesting stage (Ortez et al., 2019), and there were also strong relationship between nitrogen content

Abbreviations: Ci, intercellular CO_2 concentration; DW, dry weight; g, grams; GNP, grain number per pod; gs, stomatal conductance; GM, grain mass; GY, grain yield; J, journal; LAI, leaf area index; PN, photosynthetic rate; PNP, pod number per plant; PPFD, photosynthetic photon flux density; R2, R4 and R6, reproductive stage; TCC, total chlorophyll contents; TN, total nitrogen; E, transpiration rate; V5, Vegetative stage of five trifoliate leaf.

* Corresponding authors.
E-mail addresses: sxauywd@126.com (W. Yang), mjavedansari@gmail.com (M.J. Ansari).

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in plants and photosynthesis (Yang 2014, Bassi et al., 2018). Nitrogen (N) is a significant nutrient that is needed in large quantities (Nendel et al., 2019) and considered as key component of plant proteins (Taiz and Zeiger 2010). Nitrogen is the most deficient nutrient in the soil and more nitrogenous fertilizers are applied annually than other fertilizers (Kebene et al., 2015, Jabborova et al., 2020). Improper application and rate of fertilization severely affect the soil productivity and environmental quality (Weiner 2017). Therefore, it is imperative that nitrogen fertilization should be applied in split or when a crop requires to optimize the initial growth and yield production.

Many research scholars have extensively documented the impact of N fertilization on soybean grain yield and biomass production (Sharma et al., 2017, Bargaz et al., 2018, Foyer et al., 2018, Oliveira et al., 2019, Soares et al., 2019, Zenawi and Mizan, 2019). The main sources of nitrogen for crop production are synthetic N fertilizers and in less extent nitrogen fixing bacteria (Basu et al., 2021, Hamid et al., 2021). However, still, many farmers have a absences of the knowledge how to apply the correct amount of N fertilizer at the right time. This matter is further strengthened because of low levels of nitrogen recovery, which range of 16–45%, because of the gaseous and leakage problems in the arrangement of N_2O, N_2, NH_3 and NO^{-3} . The findings from previous investigators have shown the inconsistent results (Jeffery et al., 2006, Rafiq et al., 2010, Gai et al., 2017) and informed that higher rate of nitrogen fertilizer significantly augmented the grain yield of soybean under flooded and unirrigated conditions in mid southern USA. The outcomes from the previous investigators have shown significant important of applying nitrogen at proper time. (Hammad, 2011) stated that N fertilization should not be only selected on plant uptake but also count the physiological process, He found that applying of 250 kg N ha^{-1} in three splits (1/3N at V_2, 1/3N at V_6, and 1/3N at R_1 was the best optimal doses). Gai et al., (2017) reported that grain yield was possibly increased due to more promising environmentally friendly conditions and also explored that applying nitrogen as starter dose enhanced soybean yield in China. Ma et al. (2019) apparent that excessive amount of nitrogen fertilizer had a negative impact on crop production and environmental quality.

In General, the nitrogen recovery rate is higher for the medium and low application of N rates (Khalofah, 2021). The farmers need to apply the required quantity of nitrogen fertilizer to confirm that plants grow and mature properly, as well as to to obtain an optimum and high quality grain yield areas, where nitrogen losses and leaching are high (Bargaz et al., 2018, Zenawi and Mizan 2019). The optimum use of nitrogen fertilizer has been showing to protect the plants below stress condition and critical development periods can leading to exciting nitrogen recovery rate and best crop yield. Plants demonstrate dynamic growth with higher net photosynthsis, therefore the huge advanced in plant dry biomass accretion causing in greater grain yield. Many plant physiological developments, such as phenology, and photosynthesis, are marked by N in field conditions.

We hypothesized that adequate N fertilization is needed at critical growth stages of soybean, and that lower N input reduces photosynthetic activities, based on the findings of the above literature review, and as a result, it has an effect on soybean physiological characteristics and grain yield. However, if nitrogen inputs are too high, the soybean crop will be unable to use it effectively, resulting in a lower nitrogen recovery rate and poor environmental and economic sustainability. To match the grain yield of various crops, it is important to maximize N fertilization inputs. Some researchers only looked at the effects of nitrogen fertilization on grain production, but the results were still unsatisfactory. Furthermore, evidence-based awareness of the effects of nitrogen fertilizer on soybean growth and seed yield is needed. Hence, this current study was established to determine the effects of N fertilization rates to enhance the sustainable soybean production under winter stress environments. The objectives of this study were: (i) to examine the effects of N rates and timings on photosynthetic characteristics, total dry biomass and grain yield of the soybean, (ii) to observe the relationships of grain yield with physiological characteristics of the soybean after N fertilization.

2. Materials and methods:

2.1. Description of the experimental site

The Field experiment was carried out in Taigu county (N 37°25′, E 112°33) Shanxi Province, located in the loess plateau of China. The soil originated from loess plateau parent material was categorized as calcareous cinnamon soil with more than 30% clay in the surface soil. The soil of the investigational field was further classified as Alfisols in the USDA soil Taxonomy. The climate of experimental field was temperate and it has annual average temperature 13 °C, annual mean rainfall 442 mm, annual mean potential evapotranspiration 1840.2 mm and annual frost free period of 300 days. The soil at the experimental location contains of a clay loam texture (56% sand, 26% silt, and 18% clay), with pH 7.2, total nitrogen 51.12 g kg^{-1}, total phosphorus 19.34 mg kg^{-1}, available potassium 243.26 mg kg^{-1} and organic matter 22.23 g kg^{-1} in the soil surface.

2.2. Experimental details

The soybean plants were planted in an experimental field and repeated three times using a randomized full block template with a split-plot arrangement. Nitrogen fertilization rates (0, 75, 150, 225, and 300 kg N ha^{-1}) were used as main plots, while four N fertilization timings were used as sub-plots: V5 (trifoliate leaf), R2 (full flowering), R4 (full pod stage), and R6 (full seed). Nitrogen was applied as 40%:30%:20%:10% at four critical stages of the soybean (trifoliate leaf, full flowering, full pod stage and full seed) respectively, each nitrogen rate was applied at equal proportion. Sowing of the experiment was conducted on 4 July 2018 and harvested in 8 October 2018. The experimental plots were 25 m2 (5 m × 5 m) with 6 rows of soybeans. Soybean crop plants were spaced 40 cm apart in rows, with a plant to plant gap of 12.5 cm preserved by manual thinning, this practice was completed After 15 days of germination, establish a uniform density of soybean plants of 100,000 plants per hectare. The nitrogen supply for the experimental field was urea (46.4%), N fertilizer was applied according to the treatments by monitoring crop growth stages, and an additional 40 kg ha^{-1} was applied at sowing time in each experimental unit. At sowing time, phosphorus was added as triple superphosphate (16%) at 120 kg ha^{-1}, and potassium was applied as potassium chloride (45%) at 60 kg ha^{-1}. All the other agricultural measures such as, weed control, irrigation, disease and pesticide application were accomplished dependably and timely on the base of crop growth stage, demand, and using conventional practices of Shanxi province.

Seven soybean plants were randomly selected from each plot, collected samples were immediately taken back to the laboratory for measurement. Use an electronic balance to weigh the fresh biomass (g) of one of the soybean plants and convert it into soybean per unit area (kg ha^{-1}). After the above-mentioned soybean plants were placed in a kraft paper bag, they were placed in an oven, and the temperature was raised to 105 °C for 30 min. Then, it was then baked at 80 °C for 24 h to achieve a steady dry weight. The dry weight biomass per unit area was calculated using the weight (g) of dried soybeans (DWB, kg m^{-2}). The dry weight technique

(Feng et al., 2016) was applied to measure the dry weight biomass (DWB).

2.3. Measurements of photosynthetic characteristics

The photosynthesis characteristics, total chlorophyll and leaf area index (LAI) were measured in fully matured leaves of soybean plants. Chlorophyll was appraised in three selected flag leaves extracts at anthesis stage using the method of (Wu et al., 2014, Corassa et al., 2018). Briefly, extraction of chlorophyll was done by using acetone (95%) and impurities were detached by centrifugation. The fresh chopped leaves (0.8 g) were immersed in a 25 ml test tube containing 95% ethanol and wrapped it in the dark for 48 h till the chlorophyll was completely extracted. Chlorophyll *a* and *b* were analyzed using an ultraviolet Shimadzu UV-1800 UV spectrophotometer at 665 and 649 nm, respectively, and total chlorophyll was determined as the amount of chlorophyll *a* and *b*. A portable photosynthesis meter, the Li-6400, was used to determine photosynthetic characteristics (LI-COR, Lincoln, Nebraska, United States) in the field conditions at anthesis stage during 2017–2018 growing season. The photosynthetic parameters were determined using mature leaves from fresh soybean plants from each experimental plot. All these measurements were taken among 9:30 am to 11:30 am on a sunny day to avoid the midday decrease in photosynthesis. The leaf chamber (6 cm^2) conditions were set as flow rate of 500 μmol s^{-1} with 400 ppm CO_2 and irradiance was set at 1000 μmol (photon) m^{-2} s^{-1}. Before the measurements, a leaf sample was induced with photosynthetic photon flux density (PPFD) of 1000 μmol m^{-2} s^{-1} for 20 min. Later, the measurements such as net photosynthetic rate (P_N), internal CO_2 concentration (Ci), stomatal conductance (g_s) and transpiration rate (E) were recorded automatically by a portable photosynthesis meter *Li-6400*. Whereas the LAI was measured three times by using the SunScan canopy analysis system (Delta-T Devices, Cambridge, UK) from 10:00 to 12:00 am for all selected plant leaves in each plot.

2.4. Total N concentration

The collected soybean plants were dried for 1 h at 105 °C for tissue destruction and dehydrated at 80 °C in an oven till the constant weight was achieved. Later, each soybean plant was crushed and ground to measure nitrogen (N) contents. After digestion with concentrated H2SO4 (K2SO4 and CuSO4 as catalysts), the N content of soybean plants was determined using an automated Kjeldahl analyzer, as defined by Barbano and Clark. (1990).

2.5. Yield and yield related components

The yield contributing components were measure as number of grain per pod, pods plant^{-1}, 100-grain weight. Soybean seed yield was measured by harvesting the fifty soybean plants from each treatment, by hand collected from central rows of each plot. After all of the soybean plants had reached full maturity (95% of pods had developed pod color), the plant samples were air dried for 15 days. The dried plants were manually threshed and weighed to determine each plant's soybean seed yield, which was then translated to kg ha^{-1}. In addition, the number of pods on each plant and the number of grains per pod were measured and reported in the plants that were collected. The harvested plants' 100-grain weight was also calculated.

2.6. Statistical analysis

ANOVA was used to measure the impact of nitrogen application on soybean biomass, physiological properties, and yield elements, followed by the Tukey HSD (Honest Significant Difference) post-hoc test (significance level p£0.05). Pearson correlation was perfomed to understand the relationships among grain yield and photosynthetic characteristics of soybean plants. Linear regression was also used to signify the relationship of grain yield with biomass components and photosynthetic characteristics. SPSS version 19.0 and SAS version 9.3 were used for all statistical analysis.

3. Results

3.1. Grain yield components and N accumulation

The grain yield and yield-related components of a soybean crop grown at various nitrogen (N) rates are stated in this paper (Table 1). Grain yield exhibited a significant and positive response (*p*£0.05) to different rates of N fertilization. The difference between nitrogen rates was significant, and 225 kg ha^{-1} twisted the grain yield of soybean (Table 1). Despite the fact that various treatments showed that applying N fertilizer at 75, 150, 225, and 300 kg ha^{-1} improved grain yield by 5.35%, 6.88%, 7.73%, and 7.34% in soybean, respectively, across the control plots. The 225 kg N ha^{-1} treatment recorded the highest soybean grain yields of 3233.62 kg ha^{-1}. In the soybean crop, however, there was no major grain yield gap between 225 kg N ha^{-1} and 300 kg N ha^{-1}. Our findings revealed that the number of grains per pod, pods per plant were increased by 15.67%, 12.71%, 28.38% and 8.77% under 75, 150, 225, and 300 kg ha^{-1}, respectively as compared to control. In comparison to control, the maximum value for pods plant^{-1} 21.02 in soybean was recorded at 225 kg N ha^{-1}. N In comparison to the control, fertilization of 75, 150, 225, and 300 kg ha^{-1} raised pods number plant^{-1} by 15.67%, 12.71%, 28.38%, and 11.06% in soybean, respectively.

There was a remarkable significant (*p*£0.05) difference for 100 seeds weight in soybean under the different nitrogen rates (*p*£0.05). Treatments effects demonstrated that the N fertilization of 75, 150, 225, and 300 kg ha^{-1} increased 100 seeds of soybean by 4.71%, 3.44%, 10.18%, and 3.95%, respectively, in comparison the control (0 kg ha^{-1}). In comparison to control, the soybean had the maximum 100 seed index of 32.02 at 225 kg ha^{-1}. In our research, 225 kg N ha^{-1} had a major impact on the number of pods per plant, while 75 kg N ha^{-1} had no effect on the number of pods per plant in soybeans (Table 1). Nitrogen content in leaves significantly increased with increase of nitrogen application (Fig. 1). The average of total nitrogen content in leaves were 47.79% at V_5, 63.52% at R_2, 57.34% at R_4 and 33.62% at R_6 in the treatment of 225 kg N ha^{-1} respectively. The maximum leaf nitrogen content value was observed 58.33 at stage R_2 (flowering stage) at under N225 kg N ha^{-1} as compared to N control plots and minimum were recorded at V_5 at (vegetative stage) and R_6 at (maturity stage) were observed 48.31 under N150 and 41.24 at N75 respectively.

3.2. Photosynthetic characteristics

In this paper, the photosynthetic characteristics of soybean in various stages of development are discussed (Table 2). In comparison to the regulation, rising N fertilizer rates had a positive effect on total chlorophyll content at various stages of development. When the soybean got 225 kg N ha^{-1} at the R6 rising level, the highest chlorophyll 3.96 was observed, while the control (0 kg N ha^{-1}) had the lowest (chlorophyll) at all levels. The chlorophyll content of soybean leaves did not differ significantly between the 75 kg N ha^{-1} and 150 kg N ha^{-1} treatments (Fig. 2). In contrast to other treatments, the 225 kg N ha^{-1} treatment greatly improved the stomatal conductance (gs), transpiration rate (E), and photosynthesis rate (PN) of soybean leaves at the R_4, R_6, and V_5 levels.

Table 1
Effect of different nitrogen rates on the grain yield components of soybean crop.

N (kg ha^{-1})	Pods plant^{-1}	Number of grains pod^{-1}	100-grain weight (g)	Grain yield (kg ha^{-1})
0	18.70 ± 1.02b	2.36 ± 0.22c	29.06 ± 1.20b	3001.48 ± 49.78c
75	19.86 ± 1.07b	2.73 ± 0.15b	30.43 ± 1.28a	3162.09 ± 52.62b
150	20.32 ± 0.60a	2.66 ± 0.09b	30.06 ± 1.38a	3208.24 ± 61.50a
225	21.02 ± 0.74a	3.03 ± 0.18a	32.02 ± 0.35a	3233.62 ± 60.40a
300	20.56 ± 0.62a	2.62 ± 0.35b	30.21 ± 1.63a	3221.89 ± 60.45a

The data is the average of the three repeats. Different lowercase letters indicate the significant (p£0.05) differences among the N rates and growing stages of soybean, means with similar letters denotes the no significant different among the treatments p > 0.05 using the HSD test in SPSS

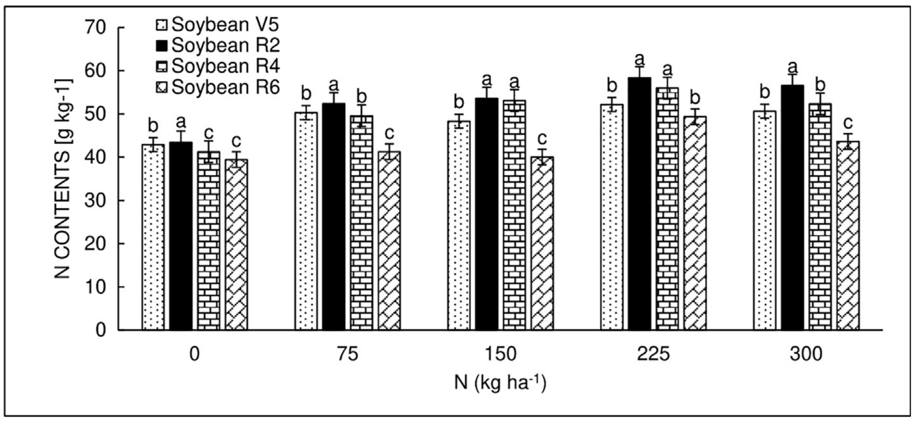

Fig. 1. Effect of different nitrogen rates and timings on N contents of soybean crop. The data is the average of the three repeats. V_5, R_2, R_4, and R_6 refer to three trifoliate, full flowering, full pod and full seeding stages of the soybean, N0-N300 kg ha^{-1} represent the nitrogen application rates. Different lowercase letters indicate the significant (*p*£0.05) differences among the N rates and growing stages of soybean, means with similar letters denotes the no significant different among the treatments *p*£0.05 using the HSD test in SPSS. Statistical bars indicate ± standard errors, (n = 3).

Table 2
Effect of different nitrogen rates and timings on photosynthetic characteristics of soybean crop.

N (kg ha^{-1})	Photosynthetic rate (µmol m^{-2} s $^{-1}$)				Stomatal Conductance (mol H_2O m^{-2} s^{-1})				Transpiration rate (mmol H_2O m^{-2} s^{-1})				Intercellular CO_2 Concentration (µmol CO_2 m^{-2} s^{-1})			
	V_5	R_2	R_4	R_6	V_5	R_2	R_4	R_6	V_5	R_2	R_4	R_6	V_5	R_2	R_4	R_6
0	13.41c	14.34c	16.57c	18.38b	0.16d	0.28c	0.22d	0.25d	2.54c	2.11d	1.59c	0.92c	222.64c	239.85c	249.46c	265.08d
75	15.62b	16.22b	17.41b	17.14c	0.22c	0.25d	0.27c	0.38c	2.98b	2.61c	1.33d	1.21b	239.04b	265.76b	256.61c	279.41c
150	17.16a	16.15b	20.08a	18.28b	0.32b	0.32b	0.32b	0.32c	2.99b	2.91b	1.35d	1.19b	247.58b	260.98b	261.81b	290.41a
225	17.71a	18.72a	21.10a	20.75a	0.36a	0.42a	0.42a	0.47a	3.54a	3.28a	2.71a	1.31a	258.31a	278.22a	269.09a	291.59a
300	16.90b	17.5a	17.75b	18.27b	0.22c	0.35b	0.26c	0.41b	2.92b	3.03a	2.54b	1.25a	256.11a	261.12b	265.28a	280.75b

The data is the average of the three repeats. V_5, R_2, R_4, and R_6 refer to three trifoliate, full flowering, full pod and full seeding stages of the soybean, N0-N300 kg ha^{-1} represent the nitrogen application rates. Different lowercase letters indicate the significant (*p*£0.05) differences among the N rates and growing stages of soybean, means with similar letters denotes the no significant different among the treatments *p*£0.05 using the HSD test in SPSS

However, at the R_6 level, the 150 N treatment had the highest intercellular CO_2 concentration (Ci). Photosynthetic rate of soybean was extremely significant due to higher CO_2 levels and reduction in photosynthetic rate by 17.71 mmol m^{-2} s^{-1} of soybean under V_5 stage. Soybean plants in normal and humid levels demonstrate significantly higher photosynthesis rate of 21.10, 20.75 mmol m^{-2} s^{-1} at R_4 and R_6 growth stages (*p*£0.05). The fertilization of 225 kg N ha^{-1} in soybean significantly increased the g_s (0.47 H_2O mol m^{-2} s^{-1}) at R_6 growing stage (Table 2). Moreover, the g_s in the leaves of soybean crop significantly improved with the reduction in the growing period (from V_5 to R_2). Plants of soybean grown up under elevated CO_2 had lower *Ci* 247.58 and 239.04 µmol mol^{-1} at V_5 stage under 150 kg N ha^{-1} and 75 kg N ha^{-1}. However, the *Ci* of soybean crop was higher than control at R_2, R_6 soybean growing stages (Table 2). Transpiration rate was quite low in soybean plants under elevated CO_2 by 1.19 mmol H_2O m^{-2} s^{-1} at 150 kg N ha^{-1} under R_6 stage of soybean. It is remarkable that *E* under 225 kg N ha^{-1} treatment was significantly increased from at V_5 3.54 mmol m^{-2} s^{-1}, while 1.31 mmol H_2O m^{-2} s^{-1} in R_6 stage as compared to the control.

3.3. Total dry biomass and leaf area index

Total dry biomass (g) and leaf area index (LAI) of soybean crop showed significant (*p*£0.05) differences from R_4 to R_6 growth stages under progressively increasing rate of nitrogen (Figs. 2 and 3). The nitrogen application significantly enhanced the dry biomass and leaf area index of the soybean at all growing stages. N application at the rate of 150 kg N ha^{-1} improved the total dry biomass by 20% at R_4 stage as compared to control (Fig. 4). However, leaf area index was improved under the 225 kg N ha^{-1} fertilization in R_4 growing stage. Moreover, the average highest total dry biomass 64.14 g, 52.20 g and 58.73 g, 51.27 g were measured under R_4 and R_6 growth stages at 225 kg N ha^{-1} and 150 kg N ha^{-1}, respectively, while highest average leaf area index was 4.22, 5.53 and

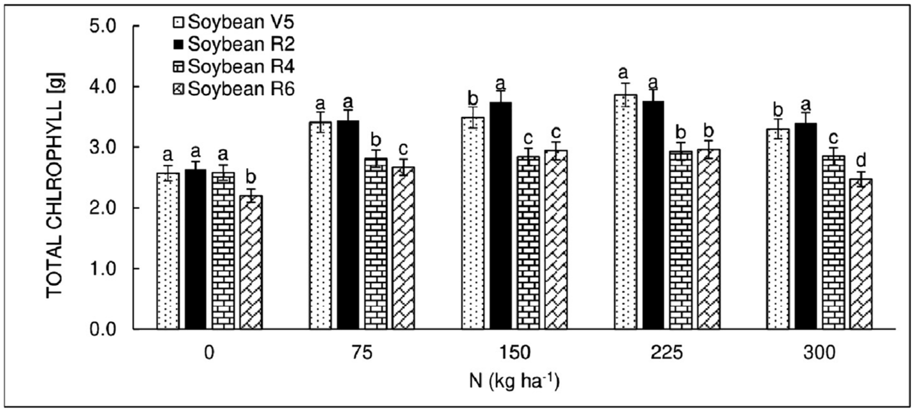

Fig. 2. Effect of different nitrogen rates and timings on total chlorophyll contents of soybean crop. The data is the average of the three repeats. V5, R2, R4, and R_6 refer to three trifoliate, full flowering, full pod and full seeding stages of the soybean, N0-N300 kg ha^{-1} represent the nitrogen application rates. Different lowercase letters indicate the significant (*p*£0.05) differences among the N rates and growing stages of soybean, means with similar letters denotes the no significant different among the treatments *p*£0.05using the HSD test in SPSS. Statistical bars indicate ± standard errors, (n = 3).

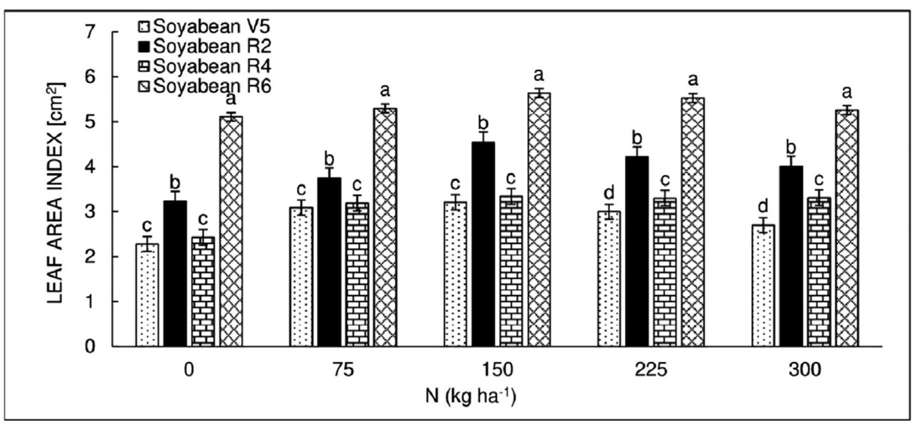

Fig. 3. Effect of different nitrogen rates and timings on leaf area index of soybean crop. The data is the average of the three repeats. V_5, R_2, R_4, and R_6 refer to three trifoliate, full flowering, full pod and full seeding stages of the soybean, N0-N300 kg ha^{-1} represent the nitrogen application rates. Different lowercase letters indicate the significant (*p*£0.05) differences among the N rates and growing stages of soybean, means with similar letters denotes the no significant different among the treatments *p*£0.05using the HSD test in SPSS. Statistical bars indicate ± standard errors, (n = 3).

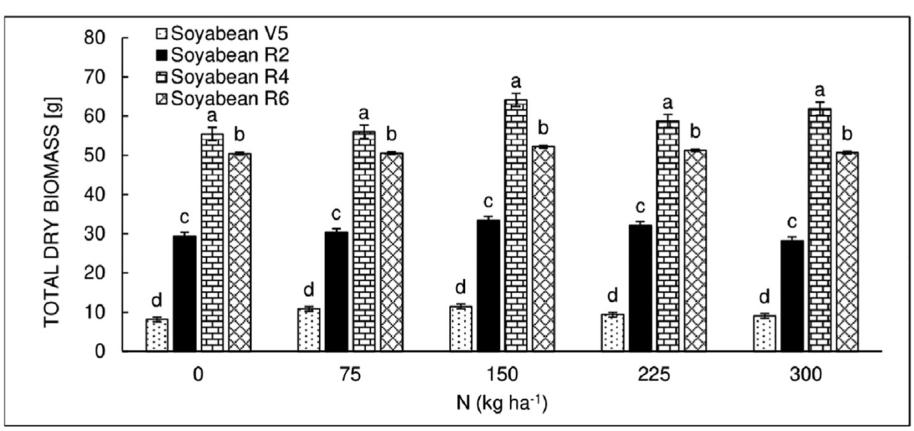

Fig. 4. Effect of different nitrogen rates and timings on total dry biomass of soybean crop. The data is the average of the three repeats. V5, R2, R4, and R6 refer to three trifoliate, full flowering, full pod and full seeding stages of the soybean, N0-N300 kg ha^{-1} represent the nitrogen application rates. Different lowercase letters indicate the significant (*p*£0.05) differences among the N rates and growing stages of soybean, means with similar letters denotes the no significant different among the treatments *p*£0.05 using the HSD test in SPSS. Statistical bars indicate ± standard errors, (n = 3).

4.55, 5.64 in R_2 and R_6 stage under 225 kg N ha^{-1} and 150 kg N ha^{-1}, respectively.

3.4. Relationship of grain yield with total dry biomass, yield components and chlorophyll

In this study, grain yield of soybean crop presented a positive significant relationship with stomatal conductance and total N content (r^2 = 0.36), (r^2 = −0.41), respectively (Figs. 5 and 6). The maximum higher relationship of grain yield was noted with 100 seed weight (r^2 = 0.41) and of photosynthetic rate was observed with intercellular CO_2 (r^2 = 0.31). Similarly, linear regression analysis confirmed that seed yield of soybean was positively and negatively associated with total chlorophyll and photosynthetic rate (r^2 = 0.20), and (r^2 = 0.24), respectively, highest correlation coefficient was observed with total N content and 100 seeds weight (r^2 = 0.41) and (r^2 = 0.35), respectively. Moreover, the linear regression analysis also confirmed that seed yield had no significant relationship with 100-grain weight and leaf area index of the soybean crop.

4. Discussion

In this study, grain yield of soybean revealed a significant response to different rates and timings of nitrogen fertilization, 225 kg N ha^{-1} donated a significant progress in the grain yield and yield components. Uncomplimentary growing environments and exhaustive shading negatively impacted the soybean grain yield (Tilman et al., 2011, Lehmann et al., 2013, Soares et al., 2019), although suitable application timings and nitrogen rates significantly augmented the gain yield of soybean crop (Hu and Wiatrak 2012, Henchion et al., 2017). The enhanced grain yield of soybean crop under R_6 stage might be recognized with higher leaf area index (Fig. 3), photosynthetic characteristics, and total dry biomass production. In relation to our study, recent studies also suggested that yield of several crops improved worldwide due to sustainable application of N along with appropriate management practices (Rafiq et al., 2010, Serrago et al., 2013, Slafer et al., 2014, Liu et al., 2018). Our finding are in consistent with (Jeffery et al., 2006, Gai et al., 2017), who reported that increase in the bulk of the grain yield of soybean crop may resulting from

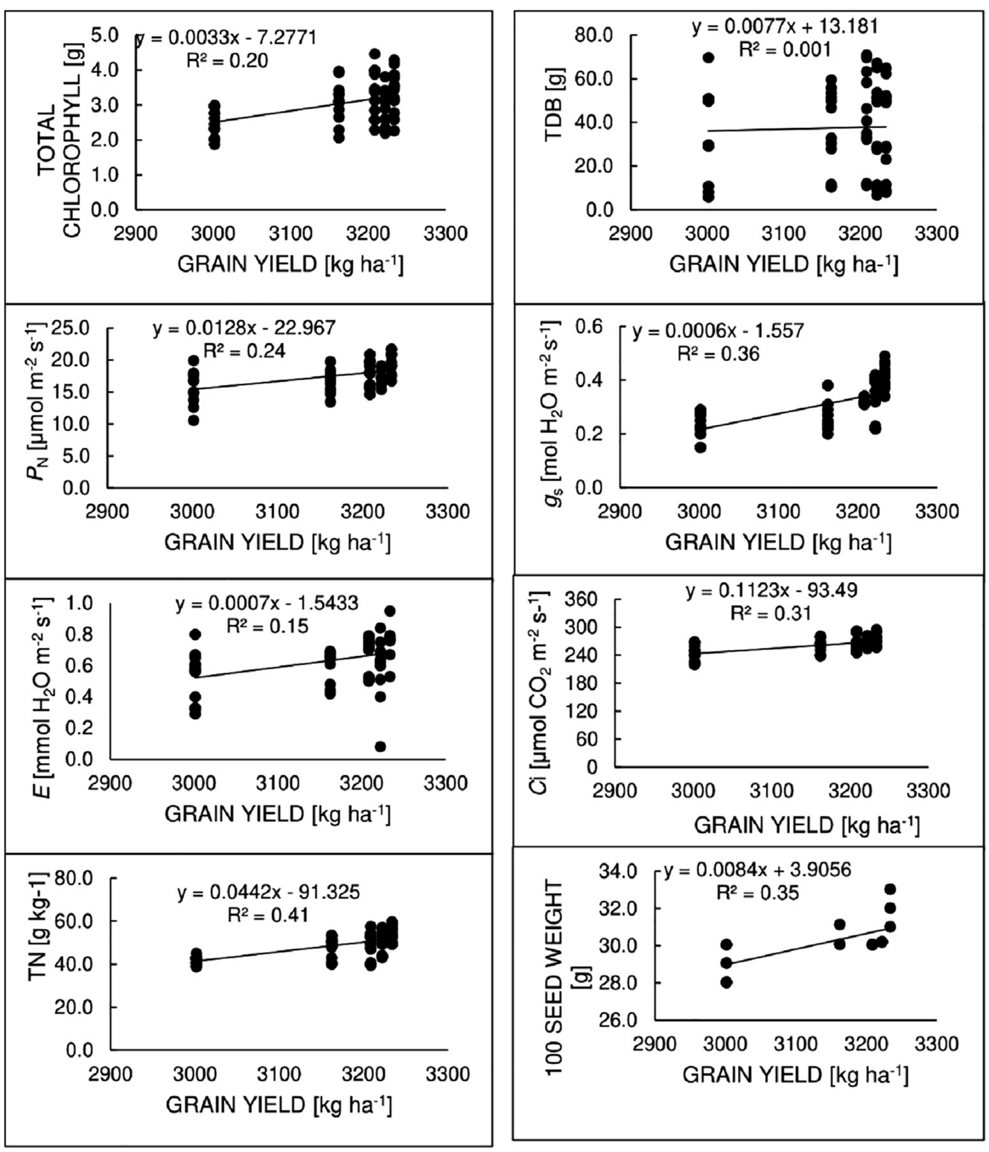

Fig. 5. Relationship of grain yield with photosynthetic traits and yield components of soybean. Total dry biomass (TDB), Photosynthetic rate (P_N), Stomatal Conductance (g_s), Intercellular CO_2 Concentration (*Ci*), Transpiration Rate (*E*), Total Nitrogen (TN), 100 grain weight (g).

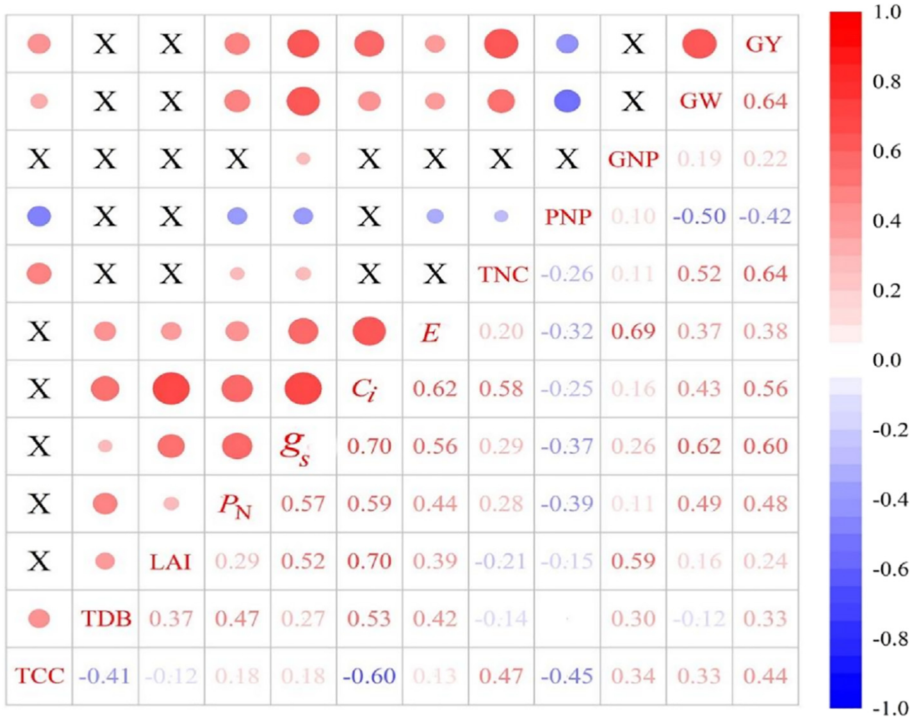

Fig. 6. Correlation coefficient of grain yield with other growth and photosynthetic traits. Grain yield (kg ha^{-1}) (GY), Pod number per plant (PNP), Grain number per pod (GNP), 100 grain weight (g) (GW), Total chlorophyll contents (TCC), Total dry biomass (TDB), Leaf area index (LAI), Photosynthetic rate (P_N), Stomatal Conductance (g_s), Intercellular CO_2 Concentration (*Ci*), Transpiration rate (*E*), Nitrogen Contents (NCS). Correlation matrix with numeric values and their graphical representation. Orange bubbles show significant relation, Blue bubbles show negative relation, white color values are less 0.01 so values are disappear and values not significant (p > 0.05 level) are marked with an X.

proper application of N fertilizer when crops needs N at suitable stage. Number of pods per plant is a yield component that is easily influenced by variations in the number of pods per plant, as well as changes in environmental and cultural condition (Rahman and Hossain 2011, Carciochi et al., 2019). Another reason for this study could be the higher rate of N at proper stage resulted greater yield because of heavier dry biomass, more photosynthetic and greater assimilation of N produced more yield and yield components.

In our current research, increased photosynthetic rate (PN) and chlorophyll (Chl) traits of the soybean crop were observed, which may be attributed to optimum temperature and precipitation during the soybean crop's increasing stages. Photosynthetic rate was affected by nitrogen fertilization and properly consumption of N significantly increased the P_N in soybean crop (Wan et al., 2013, Yang et al., 2017, Ma et al., 2019). In this study, N timings and rates considerably enhanced the P_N of soybean crop under 225 kg N ha^{-1} at V_5 and R_4 stages (Table 2). The higher PN rate was estimated in growing stage R_4, which was closely related to the higher transmittance at early growing stages, which supported soybean crop in rising their leaf area and accumulating more sunlight. This may be attributed to favorable weather and higher chlorophyll levels. (Ahmed et al., 2015), leaf area index (Wu et al., 2017), and photosynthetic rate (Yang et al., 2018) of soybean plants. Moreover, the selection of proper application timing considerably enhanced the leaf area index soybean plants. Whereas, the reduction in photosynthetic rate of soybean at control attributed to decrease the leaf area and nitrogen accumulation (Chaudhary et al., 2008). The findings of this study have implications for agronomic and environmental aspects of nitrogen fertilization management. Chlorophyll is a key component of photosynthesis, which allows the soybean crop to consume solar energy (Gai et al., 2017). The photosynthetic rate is an important feature of photosynthesis, and increasing it could increase the grain yield of the soybean crop (Wan et al., 2013). This rise in soybean crop PN rate may be related to the optimum temperature during the growing stages. This could also be concerned with the favorable environmental conditions and higher chlorophyll contents (Ahmed et al., 2015, 2018, Yang et al., 2018). Chlorophyll is a critical pigment of photosynthesis and good indicator of leaves functions under the harmful influences of different environmental agents (Menza et al., 2017), it is crucial parameter for observing the uptake of nitrogen in soybean crop (Skudra and Ruza 2017).

Our results revealed that diverse rates and timings demonstrated a significant positive effect on total dry biomass and the values of leaf area index that could be due to capturing of sunlight by soybean crop. We observed that effect was more obvious in soybean crop under R_4 growing stage as compared to other growing stages of soybean crop. Shading environmental factor can also constrains the growth of leaf by monitoring the cell proliferation in soybean crop (Wu et al., 2017). Furthermore, an improvement in the leaf area index during the R_6 growth stage indicates the optimum leaf expansion, which aids in better sunlight capture and use (Fig. 3). This arrangement may be attributed to the soybean crop's reduced canopy and senescence of mature leaves (Chen et al., 2010). In contrast to the monitor, soybean crops with nitrogen applications of 150 and 225 kg ha^{-1} had a higher leaf area index, which could be due to a longer period of green leaf area and delayed leaf senescence (Moreira et al., 2017).

The relationships of grain yield with different traits has been shown in (Figs. 5 and 6). We found positively significant relationship of grain yield with total N content, and stomatal conductance, correlation coefficient varies between (r^2 = 0.36 and 0.41), indicating that an increase in total biomass and photosynthetic parameters can support to improve the seed yield of soybean crop. These finding were in consistent with the work reported by Zhang et al. (2013). Though, the significant positive association was not establish among grain yield with leaf area index and 100-grain weight of the soybean crop. While, Moreira et al. (2017) informed that there was a positively significant relationships of soybean grain yield with leaf area index. Moreover, we found weak positive relationship of grain yield with intercellular CO_2 (r^2 = 0.31) and relationship with 100-grain weight (r^2 = 0.41). Positive significant relationships between photosynthetic traits were also observed in our study. Our findings are in agreement with some investigators who stated a significant relationship between chlorophyll, photosynthetic rate and chlorophyll traits (Chen et al., 2010, Zhang et al., 2013, Ahmed et al., 2015, Gai et al., 2017, Skudra and Ruza 2017, Wu et al., 2017, Yang et al., 2018). A important positive association between chlorophyll content and grain yield was also discovered by (Shoaib et al., 2018).

5. Conclusions

Timing of nitrogen (N) fertilizer application markedly impacted on the photosynthetic parameters, total dry biomass and seed yield of soybean. N accumulation in soybean was improved as the application of N was better corresponding with the plant N demand at ideal growing stage. Nitrogen application significantly increased the soybean photosynthetic rate, dry biomass, leaf area index, intercellular CO_2 concentration, stomatal conductance under the developmental stage (R_2, R_4, and R_6) of soybean at 225 kg N ha^{-1}. Moreover, the grain yield of soybean were observed to be positively and significantly associated photosynthetic parameters and total dry biomass. To identify the optimum and sustainable rate of N fertilization at proper growing stage is the effective approach to enhance the grain yield by the selection of optimum N timings and N rates.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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