EFFECT OF SHADE TREATMENTS ON MORPHOLOGY, PHOTOSYNTHETIC AND CHLOROPHYLL FLUORESCENCE CHARACTERISTICS OF SOYBEANS (*GLYCINE MAX* L. MERR.)

KHALID, M. H. B.[1†] – RAZA, M. A.[2†] – YU, H. Q.[1†] – SUN, F. A.[1] – ZHANG, Y. Y.[1] – LU, F. Z.[1] – SI, L.[1] – IQBAL, N.[2] – KHAN, I.[3] – FU, F. L.[1] – LI, W. C.[1*]

[1]*Maize Research Institute, Sichuan Agricultural University, Chengdu 611130, PR China*

[2]*College of Agronomy, Sichuan Agricultural University, Chengdu 611130, PR China*

[3]*Department of Grassland Science, Sichuan Agricultural University Chengdu 611130, PR China*

[†]*These authors contribute equally to this work.*

**Corresponding author*
e-mail: aumdyms@sicau.edu.cn; phone: + 86-159-28662654; fax: + 86-028-86290960
address: Sichuan Agricultural University, NO. 211, Huimin Road, Wenjiang District, Chengdu, Sichuan 611130, China

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Abstract. In maize soybean intercropping-system maize plants significantly increase the shade density at soybean canopy, and few people studied the threshold level of shade for sustainable production of soybean in this system. This experiment was started to determine the effect of four different shade treatments T_{75} (75%); T_{50} (50%); T_{25} (25%); T_0 (0%, control) on morphology, physiology and yield of soybean plants. Relative to T_{75}, treatments T_{25} and T_0 significantly increased the stem diameter and stem breaking strength while plant height was decreased. The chlorophyll a, chlorophyll b, and chlorophyll a + b, photosynthetic and chlorophyll fluorescence characteristics were improved as shade decreased, and maximum values were observed in T_{25} and T_0. Similarly, enzymatic activity of Rubisco was accelerated from T_{75} to T_0. However, genes related to sucrose synthesis (SS and SPS) were down-regulated by increasing shade (T_{75}). Importantly, non-significant differences were measured for seed-yield between T_{25} and T_0, and the plants of soybean under T_{25} produced 88% of T_0 yield. Overall, these results implied that agronomist should develop an appropriate intercropping planting pattern where the maximum shade density ranges from 20 to 30 % to obtain higher seed yield of soybean crop under intercropping-system.
Keywords: *Rubisco, intercropping, chlorophyll fluorescence characteristics, sucrose synthesis, seed-yield*

Introduction

Sun radiations are among the most important abiotic factors for agricultural production (Yang et al., 2014, 2018a). For most crop plants, even a slight increase or decrease in light intensity leads to considerable changes in photosynthetic characteristics (Wu et al., 2017). Light intensity affects the central processes of crops such as physiology, biochemistry and cell division (Kong et al., 2016; Yang et al., 2018b; Wu et al., 2018). Indeed, the numerous plant processes impair with decreasing light intensity which bring dramatic developmental and physiological changes, leading to a rapid decrease of these processes (Yang et al., 2015; Wu et al., 2016). Shading conditions could affect carbon balance of crop plant because the carbohydrate (sugars) demand increases while its production decreases: rates of physiological processes rise while the photosynthetic yield reduces (Yang et al., 2018a). Accordingly, tolerance to

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shade stress reduced at low photosynthetic rate in C_3 plants (Su et al., 2014). Moreover, the pattern of carbohydrates (Sugars) into expensive processes, like the biosynthesis of defense proteins (notably light-harvesting chlorophyll protein) raises with increasing shade (Yang et al., 2018a). The photosynthetic rate is the major driver of crop plant carbon balance, optimum and continuous availability of light should also be considered into account to study the plant responses to shade stress.

Crop growth as dry matter production is largely dependent on current photosynthesis and, therefore, one of the main important changes by shade stress in crop growth is ascribed to its huge reduction of net photosynthesis (Yang et al., 2018b). Reductions in photosynthesis could occur thanks to two main principle mechanisms (Yang et al., 2017): (i) decrease CO_2 diffusion into leaves, since the decrease internal and stomatal conductance (g_i and g_s, respectively), and (ii) metabolic potential inhibition for photosynthesis by inhibiting the leaf growth and enlargement by controlling the cell proliferation (Wu et al., 2017, 2018). However, further investigations are needed due to the relative significance of such mechanisms is debatable. The amount and activity of important enzymes involved in CO_2 fixation and regeneration of rubisco-1, 5-bisphosphate (RuBP) determined the metabolic potential of photosynthesis in plants under different conditions (Seemann and Sharkey, 1986; Delfine et al., 1999; Redondo-Gómez et al., 2007) as well as the activity and content of light capturing components, electron transport fragments, and energy transferring enzymes (Kao et al., 2003; Ranjbarfordoei et al., 2006; Stepien and Klbus, 2006). In photosynthesis Rubisco (RuBP carboxylase or oxygenase) catalyzes the process of CO_2 fixation (Mauser et al., 2001), which is directly involved in the first phase of Calvin Benson cycle and accounting for 12 to 35 percent of the leaf protein especially in C_3 crop plants (Evans and Seemann, 1989). In past reports, it has been revealed that the main biochemical restraint involved in shade-associated down regulation of net photosynthetic rate was reduction in the amount or activity of Rubisco (Evans and Seemann, 1989).

In past few years, chlorophyll fluorescence measurements have been known as an informative and useful indicator characterizing different light responses of photosynthesis. Considerable attention was paid to investigate and to determine the important characteristics of this technique (Schreiber et al., 1995). Chlorophyll fluorescence mainly and effectively used to measures the quantum yield of photosystem II and photo-inhibition by determining the potential quantum yield under prevailing light and shade conditions (Rascher et al., 2000). Shade significantly affected the performance and structure of the photosynthetic apparatus (Yao et al., 2017). It blocks the energy transport from PSII to PSI, reduces the leaf thickness, palisade and spongy tissues which results in low chlorophyll fluorescence (Wu et al., 2017; Yao et al., 2017). Thus, in this present study we aim to investigate the chlorophyll fluorescence parameters of soybean plants in response to different shading conditions because plant photosynthetic characteristics are directly dependent on the net performance of chlorophyll fluorescence parameters.

Maize (*Zea mays* L.) soybean (*Glycine max* L. Merr.) intercropping system is the main planting pattern for cereal and legume production (Rahman et al., 2017). Within the maize-soybean intercropping system – described here as the deliberate sowing of soybean crop within the intercropping strip area of maize planting – the presence of maize crop adds a level of complexity in terms of light environment dynamics for resource-use. Importantly, intercropping system of maize and soybean is used extensively in many parts of China and farmers are achieving a land equivalent ratio of

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1.3-1.4 that is much higher than other relay-intercropping systems in the world (Liu et al., 2018). However, soybean plants are extremely sensitive to shading conditions (Wolff and Coltman, 1990; Feng et al., 2018) and in this system soybean plants suffered from severe maize shading during their vegetative growth period from germination to maturity that increased the seedling height of soybean plants and it became more susceptible to lodging as the intensity of shade increases (Li et al., 2014a,b). Physio-morphological responses of soybean plant under shade conditions have also been considered. A number of studies have shown the significant reduction in stomatal conduction, photosynthetic rate, number of nodes, number of seeds and eventually seed yield (24~50%) under shade conditions (Yang et al., 2017). Therefore, great emphasis is placed on crop management with an aim to make plants more efficient in light use and enhance yield under shade conditions. However, the comprehensive understanding of physiology, morphology and gene expression related to carbon metabolism (SS and SPS) is still unclear because they are highly influenced by environmental factors under changing conditions especially intercropping systems.

To investigate the role of shading in regulation of soybean morphology and reproductive development, studies of shade treatments on soybean plants were carried out to (i) determine the impact of different shade treatments on morphological characteristics of soybean plants, (ii) study the effect of different shade treatments on photosynthetic and chlorophyll fluorescence parameters of soybean plants, and (iii) to suggest the threshold level of light intensity required by soybean plants for utilizing the available resources adequately to produce higher soybean seed yield under intercropping conditions. Overall, the main objective of this paper is to provide information for agronomists to develop appropriate planting patterns in which soybean plants receive optimum light intensity for their growth.

Materials and Methods

Plant Material and Growth Condition

Pot experiment was conducted at the greenhouse characterized by a 12 h dark/12 h light photoperiod, 28°C day and 25°C night temperature and approximately 60% relative humidity (in 2018). Experiment was complete randomized design with shade tolerant cultivar of soybean (*Glycine max* L.) Nandou-12 bred by NAAS (Nanchong Academy of Agricultural Sciences) was selected from 30 soybean varieties recognized on the basis of shade tolerance. The ten seeds were sown in pots with dimension of (30 cm diameter, 20 cm height). Soil was collected from the tillage layer of 0-10 cm depth at farm land located in Wenjiang, China. Soil textural class is silty clay loam or silty clay. Chemical properties were: 29.0 g kg^{-1} of SOC, 2.10 g kg^{-1} of total N, 0.78 g kg^{-1} of total P, 16.0 g kg^{-1} of total K, 154 mg kg^{-1} of available K and 7.11 of pH (1 : 5 v/v). The soil was air-dried and sieved through a 4-mm sieve. Basal nutrients were applied at the following rates (mg kg^{-1}): 210 urea, 215 KH_2PO_4, 160 $CaCl_2.2H_2O$, 45 $MgSO_4.7H_2O$, 8 Fe-EDTA, 7 $ZnSO_4$ and 4 $CuSO_4$, 0.8 H_3BO_3, 6.8 $MnSO_4.H_2O$, 11 $ZnSO_4.7H_2O$, 3 $CuSO_4$ $.5H_2O$, 0.5 $CoSO_4.7H_2O$, 0.4 $Na_2MoO_4.2H_2O$. All the plant-nutrients were mixed thoroughly with sand and soil. After the germination of soybean seeds, three plants per pot were maintained up to maturity and every treatment had 15 pots. In addition, when the first trifoliate leaves developed the soybean seedlings were transferred to four different shade treatments (Shao et al., 2014): T_{75} (75% shade); T_{50} (50% shade); T_{25} (25% shade); T_0 (0% shade, normal sunlight) and the shaded

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conditions were provided by a covering the pots with black nylon net. The experimental period duration was 110 days. Plants were harvested at maturity and seeds were air-dried for seed yield and yield related parameters.

Measurements and Sampling

Plant Morphological Traits

Plants were collected from each replicate for morphological measurements, the plant height; stem diameter and plant biomass were measured at stages V_4 (four trifoliate of vegetative growth), R_1 (flower initiation at the start of reproductive growth) and R_5 (seed filling at the start of seed formation) of soybean because these are the critical stages which determines the growth and development of soybean. All the plant samples of soybean plants were placed to 105°C for 1 hour and dried to constant weight at 75°C plant biomass. Leaf area was measured by using (CI-203 CID Bio-Science Portable Instruments for Precision Plant Measurement Inc. 1554 NE 3rd Ave, Camas, WA USA). The basal internode (first internode) was used to determine the stem breaking strength of soybean plants by using the digital plant lodging tester (YYD-1, Zhejiang Top Instrument Co. Ltd., Hangzhou, China) according to the previously described method (Liu et al., 2016).

Photosynthetic Pigment Concentration

Latest fully expanded leaves from each treatment were sampled. Chlorophyll (Chl a, Chl b, and Chl a + b) was extracted by grinding samples with chilled mortar and pestle in 10 ml of 80% acetone and centrifuged 1000 rpm for 3 min at 4°C. The samples were placed in ice-cold 10 ml of 80% aqueous acetone solution in the dark for 24 h at room temperature. The supernatant was then separated and analyzed by spectrophotometer (UV-2250, Kyoto, Japan) at wavelengths of 663, 645, and 470 nm as described by Fan et al. (2018) to evaluate the Chl a, Chl b, and Chl a + b content, respectively (Fan et al., 2018).

Photosynthesis

Three fully expanded leaves of soybean plants from each treatment were selected at V_4, R_1 and R_5 stages of soybean. Photosynthetic parameters including net photosynthetic rate (P_n), transpiration rate (T_r) and intercellular CO_2 concentration (C_i) were measured with the help of portable photosynthesis system (*Model LI-6400, LI-COR Inc.,* Lincoln, NE) between 10:00 and 11:00 am. The following settings were used: PARi = 1,000, stomatal ratio = 0.5, flow = 500 µmol mol^{-1} and reference CO_2 concentration = 400 µmol mol^{-1}.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence measurement was taken by using Fluor Technologia operated using the Fluor Images software. Fully expanded leaf samples from each treatment were taken and immediately preserved in plastic bags and placed in ice box preventing from the direct light. Then by using above mention software, samples were passed to fluorescence analyzing device. We examined maximum quantum yield (Fv/Fm), effective quantum yield of photosystem (ϕPSII), photochemical quenching (qP) and electron transport rate (ETR) by placing 20 min under light and dark

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conditions. Previously published method was followed by using FluorImager software, Technologia LTD version 2.2.2.2 (Pan et al., 2017).

Rubisco Analysis

For the measurements of Rubisco activated enzyme, frozen leaf samples (1 g) of soybean plants were ground with a mortar and pestle in an ice box and 2 ml of extraction buffer solution was used. The extracted solution was centrifuged 7000 rcf at 4°C for 15 min. Double antibody sandwich method was used to determine the level of plant Rubisco activase (RCA).The Rubisco activase (RCA) antibody was encapsulated by the micropore plate to form solid phase antibody, which was added to the micropore of the monoclonal antibody successively. 40 μl of sample diluent was added first and then 10 ml of sample solution in the micropore plate. The micropore plate was sealed with a plastic film for incubation at 37°C for 30 min and this incubation was repeated 5 times. Body - antigen - enzyme - labeled antibody complex, after thorough washing and substrate TMB color. TMB is transferred under the catalysis of HRP enzyme. Turn it into blue and turn it into the final yellow color under the action of an acid. After adding the stop solution, the absorbance (OD value) was measured within 15 min at 450 nm wavelength by enzyme marker and the sample was calculated by standard curve. The Rubisco activity was expressed as U/g (Seemann, 1989).

Biomass Accumulation and Distribution

For plant biomass accumulation and partitioning in different soybean plant organs, six soybean plants of two pots, were destructively sampled at V_4, R_1 and R_5 stages of soybean. Then all the collected soybean plants were separated into root, stem, leaves, and pod, and placed in oven for one hour at 105°C to kill the fresh-tissues and then dried the samples at 65°C to measure the constant weight before weighing of each plant organ of soybean for total biomass accumulation and partitioning analysis.

Carbon Status and Real-time Quantitative PCR Verification

To measure the starch and soluble sugar content soybean leaves were collected at V_4 stage at the end of the day and analyzed by following the previous method (Cross et al., 2006). Meanwhile, the expanding leaves of soybean plants at V_4 from all the treatments were collected for the determination of RNA abundance. All the leaves were labeled and frozen in liquid nitrogen immediately. Total RNA was extracted with RNAiso Plus (Takara, Japan). Reverse transcription and amplification of cDNA were performed using SuperScript II First-Strand Synthesis for qRT-PCR (Takara, Japan). Real-time quantitative PCR was conducted in CFX 96^{TM} Real-Time System (Bio-Rad, USA) and $2^{-\triangle\triangle CT}$ method used for data analysis.

Seed Yield and Yield Parameters

In the current experiment, all the remaining pots (9 pots, 27 plants) were collected from every treatment at maturity. All these collected sampled were utilized to measure the seed yield and yield components. All the sampled plants were dried in sun-light for seven days, threshed by hand and weighed to measure the seed yield of every treatment and then converted into g plant^{-1}. The seed number plant^{-1} was counted for all the sampled plants and average seed number plant^{-1} was calculated. Three lots of 100 seeds were collected from bulk seed lot of every treatment and dried in oven at 65°C till

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constant weight achieved, and then seed weight (mg) was measured by using an electrical balance and mean weight was calculated.

Statistical Analysis of Data

All the data recorded for every parameter was analyzed using computer software Statistix (version, 8.1. Statistix, USA) (Raza et al., 2018a). Analysis of variance (ANOVA) technique and least significance difference (LSD) test were employed to assess the effect of shading treatments on measured parameters, and all the means were compared at 5% probability level. Moreover, Microsoft Excel program was used for the graphical presentation of data using standard error (± SE).

Results

Morphological Characteristics

Different light treatments considerably changed the morphological characteristics of soybean plants at all growth stages (V_4, R_1, and R_5). *Figure 1* shows the morphological characteristics of soybean plants under different light treatments.

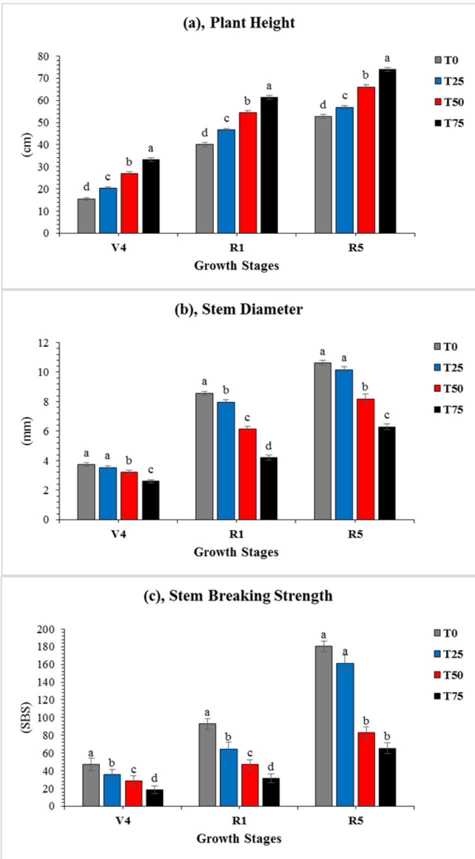

Figure 1. *Changes in plant height (a), stem diameter (b), and stem breaking strength (c) of soybean plants as affected by different photosynthetically active radiations treatments at V_4 (four trifoliate stage), R_1 (flower initiation stage), and R_5 (seed formation stage). T_{75}, T_{50}, T_{25}, and T_0 refer to 75%, 50%, 25%, and 0% shade, respectively. Means are averaged over three replicates. Bars show ± standard errors, (n = 3). Within a bar, different lowercase letters show a significant difference ($p \leq 0.05$) between treatments*

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In our study, different light treatments had a significant ($P < 0.05$) impact on plant morphological parameters, the plant height (PH) of soybean plants were substantially increased from T_0 to T_{75} at all growth stages. Specifically, the highest PH 33.2, 61.3, and 73.9 cm of soybean plants were recorded under T_{75}, and the lowest PH 15.6, 40.0, and 52.7 cm were measured in T_{75} at V_4, R_1, and R_5, respectively. Meanwhile, opposite findings were measured for stem diameter (SD) and stem breaking strength (SBS). Compared with T_{75}, SD and SBS significantly increased under T_{25} and T_0, and the SD and SBS of T_{25} and T_0, respectively improved by 41% and 60%, and 38% and 64% at R_5, than those in T_{75}. Importantly, our findings show that the shade from 25% (T_{25}) to 0% (T_0) significantly improves the morphology of soybean plants by increasing the SD and SBS.

Chlorophyll Content

In our experiment, the different light treatments significantly ($P < 0.05$) affected the chlorophyll (Chl a, Chl b, and Chl a + b) content of soybean leaves at all stages. The contents of Chl a, Chl b, and Chl a + b of soybean leaves under T_{75}, T_{50}, T_{25}, and T_0 were measured, as presented in *Figure 2*.

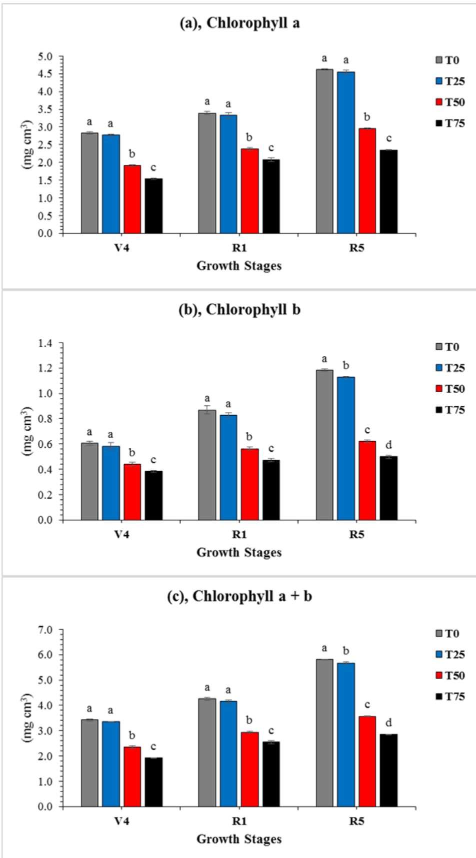

Figure 2. *Changes in chlorophyll a (a), chlorophyll b (b), and chlorophyll a +b (c) content of soybean plants as affected by different photosynthetically active radiations treatments at V_4 (four trifoliate stage), R_1 (flower initiation stage), and R_5 (seed formation stage). T_{75}, T_{50}, T_{25}, and T_0 refer to 75%, 50%, 25%, and 0% shade, respectively. Bars show ± standard errors, (n = 3). Within a bar, different lowercase letters show a significant difference ($p \leq 0.05$) between treatments*

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We observed that decreasing shade from T_{75} to T_0 increased the Chl a, Chl b, and Chl a + b contents at all measured growth stages. Interestingly, the Chl a, Chl b, and Chl a+b contents of soybean leaves were found non-significant between T_{25} and T_0 treatments, but the contents of Chl a, Chl b, and Chl a+b were always found higher under T_{25} and T_0 than those of in T_{75} treatment. Overall, at R_5, the Chl a, Chl b, and Chl a+b contents increased by 48%, 56%, and 50%, under T_{25} in comparison with T_{75}, respectively, suggesting a direct link of chlorophyll contents with the changes in available light intensity.

Photosynthetic and Chlorophyll Fluorescence Characteristics

Table 1 presents the photosynthetic characteristics of soybean plants in response to different light treatments. Relative to T_{75} treatment, photosynthetic rate (Pn) and transpiration rate (Tr) enhanced considerably from T_{50} to T_0 treatments, while stomatal conductance (Gs) and intercellular CO_2 concentration (Ci) of soybean plants were reduced at all V_4, R_1, and R_5 (*Table 1*). The Pn of T_{50}, T_{25}, and T_0 respectively improved by 12%, 31%, and 32% at V_4, 11%, 27%, and 28% R_1, and 7%, 17%, and 22% at R_5 as compared to those under T_{75}. Additionally, all the photosynthetic parameters of soybean plants at R_5 followed the same pattern to that of V_4 and R_1. Importantly, at V_4 and R_1 soybean plants exhibited the non-significant differences for photosynthetic characteristics under T_{25} and T_0, suggesting that shade of 25% to 0% was enough to maintain the optimum photosynthetic rate for better growth and development.

***Table 1.** Effect of different photosynthetically active radiations treatments on photosynthetically characteristics and enzymatic activity Rubisco of soybean plants at V_4 (four trifoliate stage), R_1 (flower initiation stage), and R_5 (seed formation stage)*

Stages	Treatments	Photosynthetic Characteristics				Rubisco
		Photosynthetic Rate (μmol CO_2 $m^{-2}s^{-1}$)	Transpiration Rate (mmol H2O $m^{-2}s^{-1}$)	Intercellular CO_2 Concentration (μmol CO_2 m^{-2} s^{-1})	Stomatal Conductance (mol H_2O m^{-2} s^{-1})	Activated (μmol CO_2 g^{-1} FW min^{-1})
V_4	T_{75}	10.17c	0.93c	275.87d	0.37a	0.17c
	T_{50}	11.53b	1.52b	316.50c	0.35a	0.22b
	T_{25}	14.68a	1.77a	319.64b	0.28b	0.27a
	T_0	15.04a	1.81a	367.10a	0.24c	0.28a
LSD		0.74	0.07	1.10	0.03	0.04
R_1	T_{75}	13.84b	1.92d	324.51d	0.63a	0.20c
	T_{50}	15.53b	2.24c	365.15c	0.56b	0.28b
	T_{25}	19.01a	2.72b	368.29b	0.47c	0.33a
	T_0	19.13a	3.11a	415.75a	0.36d	0.35a
LSD		2.51	0.12	1.10	0.02	0.04
R_5	T_{75}	18.54d	1.98d	305.70d	0.75a	0.21c
	T_{50}	20.03c	2.65c	372.33c	0.60b	0.31b
	T_{25}	22.41b	3.31b	445.7b	0.51c	0.36a
	T_0	23.85a	3.75a	456.70a	0.46d	0.38a
LSD		1.00	0.11	2.35	0.04	0.04

T_{75}, T_{50}, T_{25}, and T_0 refer to 75%, 50%, 25%, and 0% shade, respectively

The fate of absorbed solar energy in leaves of soybean at three different growth stages was investigated under changing light conditions, and shows in *Table 2*. In the present study, the chlorophyll fluorescence parameters including F_v/F_m, Φ_{PSII}, qP, and ETR were changed significantly under different light treatments. The F_v/F_m, Φ_{PSII}, qP,

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and ETR of soybean leaves in T_{25} and T_0 were found significantly higher than those under T_{75}. Moreover, treatment T_{25} and T_0, at R_5 significantly increased the chlorophyll fluorescence values of F_v/F_m, Φ_{PSII}, qP, and ETR by 4% and 5%, 17% and 21%, 11% and 13%, and 17% and 21%, respectively, as compared to T_{75} treatment, indicating that adequate light intensity (T_{25} and T_0) plays the fundamental role in improving the chlorophyll fluorescence parameters and photosynthetic capacity of soybean plants, which can help to maintain optimum growth and development under changing light conditions. In addition, the dynamics of chlorophyll fluorescence parameters at V_4 and R_1 stages under different light treatments showed the consistent pattern with those of at R_5 stage.

Table 2. *Effect of different photosynthetically active radiations treatments on chlorophyll fluorescence characteristics (Fv/Fm; quantum yield, ΦPSII; effective quantum yield of photosystem, qP; photochemical quenching, and ETR; electron transport rate) of soybean plants at V_4 (four trifoliate stage), R_1 (flower initiation stage), and R_5 (seed formation stage)*

Stages	**Treatments**	**Chlorophyll Fluorescence Characteristics**			
		ΦPSII	qP	ETR	FV/FM
V_4	T_{75}	0.21c	0.40c	92.93c	0.76b
	T_{50}	0.22b	0.42b	97.61b	0.77b
	T_{25}	0.24a	0.43b	102.00a	0.78a
	T_0	0.24a	0.45a	105.54a	0.79a
LSD		0.01	0.01	4.35	0.00
R_1	T_{75}	0.28d	0.60c	122.26d	0.76d
	T_{50}	0.30c	0.62b	130.62c	0.78c
	T_{25}	0.32b	0.64a	136.99b	0.79b
	T_0	0.33a	0.65a	143.08a	0.80a
LSD		0.01	0.01	5.40	0.00
R_5	T_{75}	0.31d	0.60c	132.46d	0.77d
	T_{50}	0.34c	0.63b	148.47c	0.78c
	T_{25}	0.37b	0.68a	160.37b	0.80b
	T_0	0.39a	0.69a	166.88a	0.81a
LSD		0.00	0.01	3.47	0.00

T_{75}, T_{50}, T_{25}, and T_0 refer to 75%, 50%, 25%, and 0% shade, respectively

Carbon status, Rubisco Activity and Gene Expression

To further study the impact of shade treatments on soybean growth, we measured the total soluble sugar and starch content of soybean root and shoot at the end of day. As expected, total soluble sugar and starch content were considerably increased with decreasing shade treatments (from T_{75} to T_0) in both root and shoot. The highest total soluble sugar content 2.45 mg g^{-1} and 6.47 mg g^{-1}, and starch content 0.37 mg g^{-1} and 0.69 mg g^{-1} were determined under treatment T_0 followed by T_{25} (total soluble sugar content 1.95 mg g^{-1} and 6.25 mg g^{-1}, and starch content 0.32 mg g^{-1} and 0.65 mg g^{-1}) in root and shoot, whereas minimum total soluble sugar and starch content were measured in T_{75} treatment (*Figure 3*). In this experiment, a significant difference in Rubisco activity was measured in all treatments. Acceleration in the activity of Rubisco occurred in all treatments from T_{75} to T_0, the amplitude of increase was higher under T_0 than T_{75}, T_{50}, and T_{25} treatments. However, non-significant differences were observed between T_{25} and T_0 treatment for Rubisco activity. Overall, the Rubisco activity of soybean plants under T_{25} was increased by 39%, 40% and 42% at V_4, R_1, and R_5, respectively

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with respect to those under T_{75} treatment. These results indicate that the activity of Rubisco under T_{25} and T_0 showed the similar trend and shade of 25% can be effective at the enzymatic activity of Rubisco. Homologues of soybean sucrose phosphate synthase (GmSPS1*T1* and GmSPS2*T2*) and sucrose synthase (*GmSS*) were selected after blast against Arabidopsis to determine gene expression under different treatments. All three genes for sucrose phosphate synthase and sucrose synthase were up-regulated with increasing light at V_4 stage. Relative to T_{75} treatment, the expression of GmSPS1T_1 and *GmSPS2T2* were increased by 0.34 and 0.42 folds in T_{25}, respectively. While the expression of *GmSS* was enhanced by 2.3 folds in treatment T_{25} than T_{75} *Figure 4*.

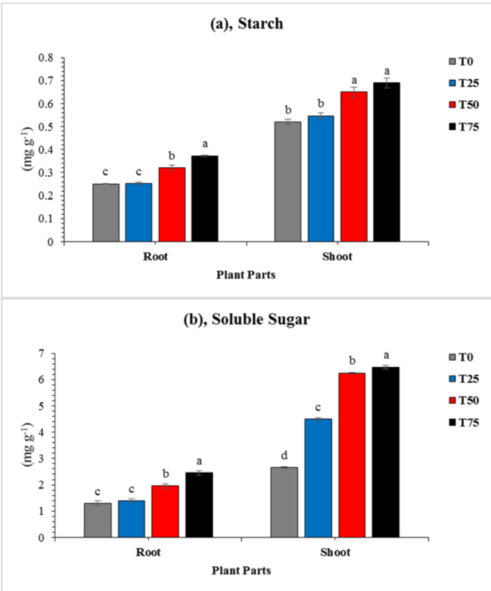

***Figure 3.** Changes in starch content (a) and soluble sugar content (b) of soybean leaves as affected by different photosynthetically active radiations treatments at V_4 (four trifoliate stage). T_{75}, T_{50}, T_{25}, and T_0 refer to 75%, 50%, 25%, and 0% shade, respectively. Means are averaged over three replicates. Bars show ± standard errors, (n = 3). Within a bar, different lowercase letters show a significant difference (p ≤ 0.05) between treatments*

Biomass Accumulation and Distribution

Changing light conditions significantly affected the total biomass accumulation (TBA) (g $plant^{-1}$) of soybean plants at various stages. In our study, the maximum TBA was 6.7, 22.4, and 35.5 under treatment T_0, while minimum TBA 4.1, 8.5, and 19.4 was observed in T_{75} treatment at V_4, R_1, and R_5, respectively (*Table 3*). However, soybean plants under T_{25} produced 86%, 76%, and 85% of the T_0 soybean biomass at V_4, R_1, and R_5, respectively. Furthermore, we also measured the biomass distribution among root, stem, leaves, and pods at different growth stages of soybean in response to changing light conditions (*Table 3*). At V_4 and R_1 stages of soybean, the maximum biomass partitioning (g $plant^{-1}$) was recorded in stem (3.8 and 13.4) followed by leaves (2.7 and 8.3) and root (0.25 and 0.65) under T_0, while at R_5 biomass distribution pattern was changes and the highest biomass was found in leaves (16.2) followed by stem (14.9), pods (2.6) and root (1.7) in T_0 treatment. Compared with T_{75} treatment, soybean plants under T_{25} and T_0 treatments, respectively obtained 40% and 35% higher pod biomass at R_5 stage.

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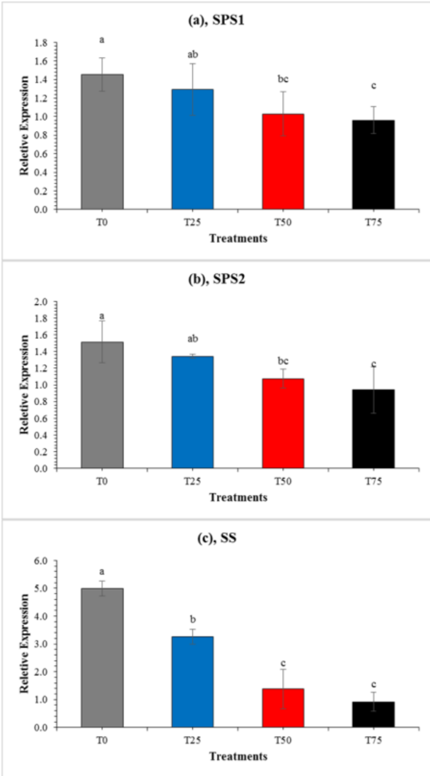

Figure 4. *Relative expression of sucrose phosphate synthase Gmsps1, (a), Gmsps2, (b) and sucrose synthase Gmss (c) genes of soybean leaves as affected by different photosynthetically active radiations treatments at V_4 (four trifoliate stage). T_{75}, T_{50}, T_{25}, and T_0 refer to 75%, 50%, 25%, and 0% shade, respectively. Means are averaged over three replicates. Bars show ± standard errors, (n = 3). Within a bar, different lowercase letters show a significant difference ($p \leq 0.05$) between treatments*

Table 3. *Effect of different photosynthetically active radiations treatments on biomass partitioning (g plant^{-1}) among different plant parts and total biomass accumulation (g plant^{-1}) of soybean plants at V_4 (four trifoliate stage), R_1 (flower initiation stage), and R_5 (seed formation stage)*

Stages	Treatments	Biomass Partitioning				TBA
		Roots	Stem	Leaves	Pod	(g plant^{-1})
V_4	T_{75}	0.19d	2.43d	1.39d	-	4.01d
	T_{50}	0.22c	2.60c	1.72c	-	4.54c
	T_{25}	0.23b	3.38b	2.20b	-	5..81b
	T_0	0.25a	3.80a	2.70a	-	6.75a
LSD		0.00	0.15	0.25	-	0.33
R_1	T_{75}	0.38c	5.16d	2.95d	-	8.50d
	T_{50}	0.41c	6.43c	4.28c	-	11.13c
	T_{25}	0.53b	10.46b	6.13b	-	17.13b
	T_0	0.65a	13.43a	8.32a	-	22.41a
LSD		0.03	0.28	0.17	-	0.42
R_5	T_{75}	0.74d	7.73d	9.33c	1.59d	19.39d
	T_{50}	0.91c	9.49c	11.27b	2.05c	23.73c
	T_{25}	1.31b	11.48b	15.04a	2.45b	30.29b
	T_0	1.68a	14.92a	16.23a	2.65a	35.49a
LSD		0.02	0.75	1.38	0.12	1.58

T_{75}, T_{50}, T_{25}, and T_0 refer to 75%, 50%, 25%, and 0% shade, respectively

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Yield and Yield Components

In our study, there was a significant impact of different light treatments on seed yield of soybean plants *Figure 5*. The highest seed yield, 15.1 g plant^{-1}, was recorded in T_0 treatment. Relative to T_{75}, soybean plants under T_{25} and T_0 produced the 22% and 31% higher seed yield. Yield parameters also varied among different light treatments. The effects of light treatments on pod number (plant^{-1}), seed number (plant^{-1}) and seed weight (mg) were significant, and pod number and seed number under T_0 treatment were found significantly higher than that in T_{75}, T_{50}, and T_{25}. Meanwhile, seed weight was found considerably heavier in T_{75} as compared to treatment T_0. Overall, light treatment T_{25} and T_0 increased the pod number and seed number by 37% and 44%, and 38% and 45% as compared to treatment T_{75}, respectively, while T_{75} treatment enhanced the seed weight of soybean seeds by 21% than T_0 treatment *Figure 5*.

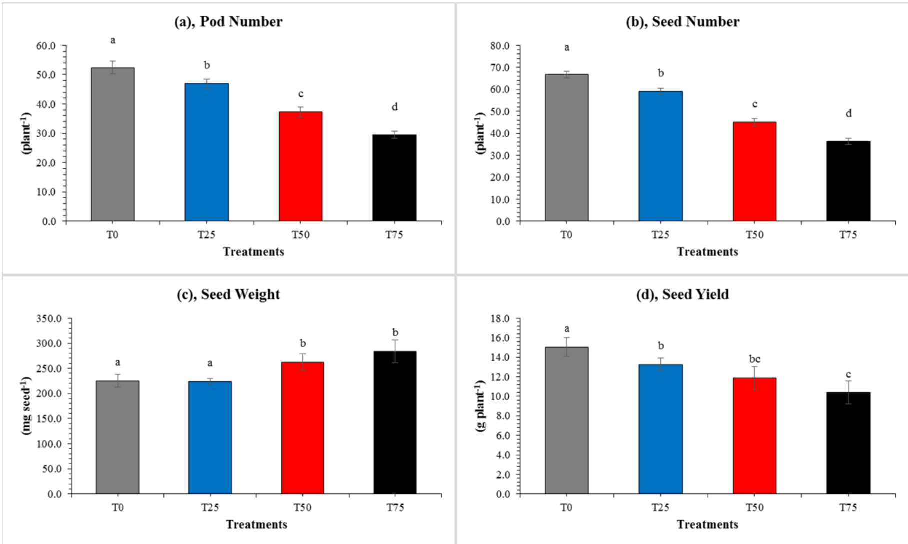

Figure 5. *Changes in pod number (a), seed number (b), seed weight (c), and seed yield (d) of soybean plants as affected by different photosynthetically active radiations treatments at* V_4 *(four trifoliate stage).* T_{75}, T_{50}, T_{25}, *and* T_0 *refer to 75%, 50%, 25%, and 0% shade, respectively. Means are averaged over three replicates. Bars show ± standard errors, (n = 3). Within a bar, different lowercase letters show a significant difference* ($p \leq 0.05$) *between treatments*

Discussion

The plant morphology has certain manipulability, and corresponding adaptation mechanisms present in changing environmental conditions (Gong et al., 2015). Use of higher planting density is the effective method for enhancing seed yields (Liu et al., 2015a). However, this practice is typically obstructed by impair light conditions (Li et al., 2014a). Numerous experiments have reported that shading conditions favor the upward growth of stem while reducing the stem breaking strength and stem diameter (Kurepin et al., 2007; Gommers et al., 2013; Yang et al., 2014). However, very few studies have Paidattention on the effect of changing shade treatments on soybean morphology to understand the threshold level of shade which will not affect the optimum soybean growth. In this study, a gradual decrease in shade (from T_{75} to T_0) significantly increased the stem diameter and stem breaking strength by maintaining the

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optimum plant height of soybean plants (*Figure 1*). These findings showed that any variation in available light at soybean canopy directly affect the morphological characteristics of soybean plants and improved light availability positively improved the soybean growth by enhancing stem breaking strength which reduced the soybean lodging (Liu et al., 2016). Importantly, soybean plants under treatment T_{25} obtained superior morphological characters than T_{75} and T_{50}, suggesting that under 25% of shade soybean plants can maintain their optimum growth. Sun-light stimulates the plant growth and development; by photosynthesis process, plants use sun-light to convert H_2O and CO_2 into carbohydrate, photosynthetic pigments (Chl a, Chl b, and Chl a+b) playan important role in changing the solar energy to chemical energy (Liang, 2000; Yuncong et al., 2007). In changing light conditions, the study of Chl a, Chl b, and Chl a+b helps as an indices for sun-light absorption (Fan et al., 2018). In past reports, researchers have confirmed that Chl contents significantly affected by changes in light availability and decrease with the reduction in light (Li et al., 2014a,b). Similarly, significant variations were measured for Chl a, Chl b, and Chl a + b contents in all shade treatments, and chlorophyll contents were enhanced from 2.4 to 4.6 mg cm^3 with the decrease in shade from T_{75} to T_0, respectively, our results are consistent with previously reported results (Wittmann et al., 2001; Fan et al., 2018). On the other hand, several scientists have claimed that Chl a, Chl b, and Chl a + b contents increase with the increase in shade, especially Chl b contents (Li et al., 2014a).

In addition to the impacts of changing light conditions on morphology and chlorophyll contents our results showed that deleterious effects of increase shade eliminated by adequate light availability (treatment T_{25} and T_0). There are many causes why soybean plants under high shade conditions produced less carbohydrate. For instance, researchers have concluded that assimilate demands of soybean plants increased while photosynthetic rate decreased under increased shade conditions (Su et al., 2014; Yang et al., 2017). In this experiment, optimum light (T_{25} and T_0) at soybean canopy led to increase the photosynthetic rate and transpiration rate while stomatal conductance and intercellular carbon dioxide levels were decreased. Therefore, this showed that the enhanced photosynthetic characteristics increased the carbon gain and improved the soybean growth (Liao et al., 2005). Moreover, these findings indicating that the increased photosynthetic rate under T_{25} and T_0 treatments may be associated with the higher chlorophyll contents (i.e. higher Chl a, Chl b, and Chl a+b contents at V_4, R_1, and R_5, (*Figure 2*) of soybean leaves (Feng et al., 2018). Improved photosynthetic capacity is always accompanied with high quantity of electrons passing through PSII (Yao et al., 2017). Chl fluorescence parameters are one of the major factors in regulation of photosynthesis and crop responses to environmental conditions because of its sensitivity and convenience (Dai et al., 2009). In addition, total soluble sugar and starch content are the direct measure of high photosynthetic rate (Iqbal et al., 2018a). Crops translocate carbohydrate from source leaves to different plant parts that decides the plants fitness under changing environments (Amiard et al., 2005). Numerous previous experiments confirmed the role of light intensity for the production of total soluble sugar and starch content in plants (Preiss, 1982; Michalska et al., 2009). Similarly, we found that the total soluble sugar and starch content were considerably improved by 33% and 144% in T_0 than T_{75}. These results were in agreement with past reports (Pilkington et al., 2015). Previous experiments have confirmed that heavy shade results in reduce photosynthesis due to the decrease in qP, PSII and ETR (Huang et al., 2011; Yao et al., 2017; Feng et al., 2018). In this experiment, similar results were

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obtained, however, improved Chl fluorescence characteristics were measured under T_{25} and T_0 treatments. These results reveals that optimum light intensity (treatment T_{25} and T_0) improves the efficiency of PSII and ETR that could enhance the photosynthetic capacity of soybean plants by improving the energy transport from PSII to PSI, our results are consistent with previously reported results (Yang et al., 2018a). The loss of rubisco activity was recognized to be very early and fast response of crop plants to shade stress (Servaites et al., 1986). While, at R_5, in current research the activity of rubisco was significantly accelerated in T_0 (0.38 μmol CO_2 g^{-1} FW min^{-1}) and T_{25} (0.36 μmol CO_2 g^{-1} FW min^{-1}) as compared to T_{75} (0.19 μmol CO_2 g^{-1} FW min^{-1}) (*Table 1*), our findings are consistent with past reports (Carmo-Silva and Salvucci, 2013). This higher rubisco activity under decreased shade treatments (T_{25} and T_0) exhibited that the higher photosynthetic rate of soybean plants directly correlated with rubisco activity under changing environments (Zhang et al., 2002).

This study provides the important data on biomass accumulation in soybean plants in different shade treatments (*Table 3*). Increased photosynthetic rate is one of the main factors for plant biomass production (Raza et al., 2018b). Previously, researchers have found that biomass accumulation is directly associated with the availability of light intensity (Kiniry et al., 2004) and reductions in light decreased the biomass production (Maddonni and Otegui, 2004). Our findings proposed that an adequate light availability (T_{25} and T_0) at soybean canopy can increase biomass accumulation by capturing and utilizing sun-light for their biochemical and physiological processes which in turn increased availability of carbohydrates for seed formation and this finding was similar to previous result (Liu et al., 2015b). Moreover, the accelerated dry matter production in soybean may be related to the utilization of major nutrients (Raza et al., 2018a) as the nutrient uptake ability of crops increased with improve light conditions (Yang et al., 2017). Additionally, we also measured the biomass partitioning in leaves, stem, pods, and seed of soybean plants in response to different shade treatments (*Table 3*). The dry matter partitioning changed considerably at V_4, R_1, and R_5 in soybean in all treatments. At V_4 and R_1, when reproductive parts were the weak sink the highest allocation of plant-biomass was determined in stem followed by leaves (Jasdanwala and Khan, 1988; Srinivasan et al., 2017). After that, at R_1 and R_5, the plant-biomass distribution changed and the most of plant-biomass assimilated to the leaves and pods in all treatments (Couch et al., 2017). Similar to our findings researchers have reported that optimum light intensity increased the assimilation of plant-biomass to economic parts, while decreased the dry matter partitioning to vegetative organs (Jasdanwala and Khan, 1988). Nevertheless, severe shading conditions (T_{75} and T_{50}) increased the retention of plant-biomass in stems (Wu et al., 2017) which did not promote the seed formation process and decreased the seed yield (*Figure 5*). These findings of plant-biomass partitioning demonstrated that accelerated translocation of photo-assimilates from the stem for pod formation occurred in soybean plants of treatments T_{25} and T_0, while severe shading conditions was not favorable to plant-biomass partitioning to pod.

Previously, it has been found that severe shading conditions significantly decreased the soybean yield and yield components (Wu et al., 2016; Iqbal et al., 2018b). Similarly, in our study, treatment T_{75} (severe shading) had significant negative effect on seed yield and yield related parameters, and minimum pod number (29.9 $plant^{-1}$) and seed number (36.4 $plant^{-1}$), and seed yield (10.4 g $plant^{-1}$) of soybean were noticed in T_{75} treatments. This is might be due to the lower photosynthetic rate and biomass accumulation, similar to our results in another study of relay-intercropping scientist has reported that soybean

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seed yield of soybean plants significantly decreased in severe shading conditions as compared to normal conditions (Yang et al., 2017). Moreover, light enrichment treatments significantly increased the pod number and seed yield of soybean. Hence, pod number (52.4 and 46.9 plant^{-1}) and seed number (66.7 and 59.1 plant^{-1}) of soybean plants might be improved in adequate light (T_{25} and T_0, respectively), these results implied that optimum light availability at soybean canopy can significantly improve the morphological parameters, photosynthetic and chlorophyll florescence characteristics which in turn considerably increased the seed yield of soybean plants by increasing the pod number and seed number.

Conclusion

The significant impacts of shade treatments on soybean have been studied previously, but rarely researchers have investigated the effects of different shade treatments on soybean plants to understand the threshold level of shade for the better growth and development. In this experiment, we showed that by selecting the shade tolerant variety soybean plants can cope shade up to 25% (T_{25}). Increased shade (T_{75}) considerably impaired the morphological characteristics (*Figure 1*), enzymatic activity of key enzyme (Rubisco) by down-regulating the important sucrose-synthase-genes (*Figure 4*). In addition, as compared to T_{75}, treatment T_0 and T_{25} significantly improved the photosynthetic and chlorophyll florescence characteristics of soybean plants especially quantum yield of PSII which in turn considerably increased the seed yield and yield-components (*Figure 5*). Overall, these results implied that agronomist should have to develop an appropriate intercropping planting pattern where the maximum shade density ranges from 20 to 30% to obtain higher seed yield of soybean crop under intercropping-system. This could be achieved by developing the narrow-wide row planting pattern in which maize plants can grow under narrow rows and soybean plants can grow in wide rows by maintaining the appropriate distance in which soybean plants can receive enough light for their optimum growth. In addition, developing the long term and environment friendly agronomic approaches to improve the seedling growth of soybean under intercropping systems by reducing the shade density in maize soybean intercropping system is an important direction for future research.

Conflict of interests. The authors have declared no conflict of interests.

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