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Effect of simultaneous shade and drought stress on morphology, leaf gas exchange, and yield parameters of different soybean cultivars

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Abstract

An interactive effect of simultaneous shade and drought stress on drought-tolerant and drought-sensitive cultivars of soybean was studied. As drought stress intensified, the net photosynthetic rate decreased in both cultivars due to reduced leaf area, relative water content, transpiration rate, stomatal conductance, chlorophyll content, and Rubisco activity which ultimately led to yield reduction. Moreover, the chlorophyll fluorescence parameters also decreased. Interestingly, a moderate shade was found helpful in alleviating the adverse effects of drought stress, specifically in resistant cultivar N12 where seed yield improved significantly under moderate drought conditions in contrast to the cultivar C103. In summary, the effect of drought stress on soybean depended on the irradiance conditions and shade could enhance soybean drought resistance, although this resistance was cultivar dependent. With appropriate cultivar selection, a moderate shade can help optimize yield and improve the performance of drought-exposed soybean.

Keywords: drought; intercropping; morphology; photosynthesis; shade; stress.

Introduction

One of the major social, economic, and scientific challenges of the modern era is the substantial increase of the human population (Tyczewska *et al*. 2018). Unfortunately, the current food production levels are not sufficient to fulfill the needs of such a large population. This scenario demands urgent consideration and adequate response to prevent devastating ripple effects. Agricultural practices such as intercropping help maximize resource use and lead to higher yield production on a given piece of land (Raza *et al*. 2019). Maize–soybean intercropping system is one of the main examples of cereal–legume intercropping (Hussain *et al*. 2019a). Specifically, maize–soybean relay intercropping is practiced largely in southwestern parts of China (Yang *et al*. 2018). The system helps in efficient utilization of farmland resources, results in low incidences of diseases, pests, and weeds, improves soil fertility, and results in higher yield and economic benefits.

Soybean [*Glycine max* (L.) Merr.], a C_3 legume, is an important oilseed crop with over 300 million tons of production globally (Sugiyama 2019). It is being produced and consumed for its protein and oil content (Song *et al*. 2016). When intercropped with maize, the crop suffers from various abiotic stresses, the most important being the shade stress (Feng *et al*. 2019, Hussain *et al*. 2020a) and moisture stress due to adjacent high stalked maize plants (Rahman *et al*. 2017, Iqbal et *al*. 2019). A combination of shade and moisture stress leads to biochemical, physiological, and structural changes at the leaf and whole plant level (Holmgren 2000, Sack and Grubb 2002, Sack 2004, Aranda *et al*. 2005).

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Abbreviations: DM – dry mass; E – transpiration rate; ETR – electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; FM – fresh mass; F_m – maximal fluorescence yield of the dark-adapted state; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; P_N – net photosynthetic rate; q_P – photochemical quenching coefficient; RA – Rubisco activity; RWC – relative water content; TM – turgid mass; Φ_{PSII} – effective quantum yield of PSII.

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Numerous previous studies have reported the negative effect of shade stress on soybean photosynthesis by blocking electron transport from PSII to PSI, thus reducing the electron transport rate, reducing the amount of ATP produced, and the amount or activity of Rubisco (Valladares and Niinemets 2008, Yao *et al*. 2017, Huang *et al*. 2018). Similarly, drought stress is also known to disrupt the process of photosynthesis mainly by altering the ultrastructure of the organelles, stomatal regulation, and concentration of various pigments and metabolites including enzymes involved in this process (Ashraf and Harris 2013). Moreover, it inhibits the photosynthesis by decreasing leaf area, photosynthetic rate per unit leaf area as well as Rubisco activity (Galmés *et al*. 2011, Basu *et al*. 2016).

Chlorophyll (Chl) fluorescence is one of the main indicators of photosynthetic regulation and plant responses to environmental conditions (Dai *et al*. 2009, Komura *et al*. 2010, Murchie and Lawson 2013). Fluorescence emission can be successfully used to monitor photosynthesis disturbances under stress conditions (Zivcak *et al*. 2014). Earlier studies have highlighted the response of Chl fluorescence to shade and drought conditions in different crops (Hussain *et al*. 2019b, Zhou *et al*. 2019, Shafiq *et al*. 2020). The decrease in Chl content is a commonly observed phenomenon under moisture (Ommen *et al*. 1999, Manivannan *et al*. 2007) and shade stress (Zhu *et al*. 2017) which is also a typical symptom of oxidative stress. In contrast, other researchers report enhanced Chl production in response to lower PAR (Terashima *et al*. 2006, Gregoriou *et al*. 2007, Lichtenthaler *et al*. 2007, Melgar *et al*. 2009) and water deficit (Estill *et al*. 1991, Hamada and Al-Hakimi 2001, Pirzad *et al*. 2011).

Drought and shade stress are important environmental factors in determining the biological yield as they affect plant productivity. Various earlier published literature provides an insight into the effect of shade and drought stress separately on soybean yield (Liu *et al*. 2003, Karam *et al*. 2005, Masoumi *et al*. 2011, Yang *et al*. 2014, 2017; Iqbal *et al*. 2018). Although great research effort has been made towards understanding the effects of shade and drought stress on soybean productivity, little attention has been given to the combined effect of both abiotic stresses. An understanding of how soybean plants respond to a combination of moisture and shade stress can play a principal role in stabilizing crop performance under stress conditions. Therefore, the present research was aimed to evaluate the interactive effect of drought and shade stress on growth, photosynthetic parameters, Rubisco activity, and yield components of the two soybean cultivars.

Materials and methods

Plant material and growth conditions: A pot experiment was conducted in 2019 in a greenhouse at the Sichuan Agricultural University, Chengdu campus, China. Seeds of two soybean genotypes, Nandou-12 (N12; shade- and drought-resistant) bred by NAAS (Nanchong Academy of Agricultural Sciences) and C-103 (C103; shade- and drought-susceptible), were used. The plants were grown in the containers (internal diameter of 17.5 cm and 17.5 cm high) filled with a mixture of soil, sand, and organic matter (5:3:2, v/v/v). The soil was taken from the Renshou experimental area. At the start of the study, the soil pH (1:2.5, soil:water) was 6.8; soil contained 20.5 g(organic matter) kg^{-1}, 1.5 g(total N) kg^{-1}, 110 mg(available N) kg^{-1}, 12.6 mg(Olsen-P) kg^{-1}, 115 mg(exchangeable K) kg^{-1}, and cation exchange capacity of 22.1 cmol$_c$ kg^{-1} of dry soil in the top 20-cm soil layer. The experiment was designed as a CRD (completely randomized design) experiment with three factors, *i.e.*, two cultivars, two light regimes, and three drought treatments. There were 12 treatments in total, each treatment had further three replications and five pots per replication were used.

Experimental scheme: The plants of both the cultivars were grown under two different light environments, *i.e.*, full light (L; PPFD = 1,000–1,200 µmol m^{-2} s^{-1} at noon) and a moderate shade (S; using single-layer black nylon net with PPFD = 500–600 µmol m^{-2} s^{-1} at noon) throughout the vegetative growth period. When plants grown in each light environment reached the V2 stage (the vegetative stage with second fully developed trifoliate leaves), they were divided into three groups; one group was watered regularly to maintain 75 ± 2% field capacity (T1; control) while water for remaining two groups of plants was withheld up till the V5 stage (the vegetative stage with fifth fully developed trifoliate leaves) to maintain drought conditions, *i.e.*, moderate drought (T2; 55 ± 2% field capacity) and severe drought (T3; 35 ± 2% field capacity). The drought levels were maintained on a mass basis, *i.e.*, gravimetrically. Moreover, the drought level in the substrate was maintained similar under both light regimes. As the reduction of water content under shade conditions was lesser, the pots were watered accordingly, whereas water loss in the pots under full light was comparatively larger, thus, more frequent watering was required to maintain the desired moisture content during the drought treatments. The data for various morphological and physiological traits were recorded at the V5 stage. At beginning of the R1 stage (the beginning of flowering), shade from all plants was removed (*i.e.*, no shade during the reproductive growth period which imitates the light conditions in the maize–soybean relay intercropping system) and all plants were watered regularly to maintain 75 ± 2% field capacity. Finally, yield data were recorded at the R7 stage (the reproductive stage with mature pods).

Morphological parameters: At the V5 stage, plants from each treatment were destructively sampled to measure various morphological parameters. The plant height [cm] was measured using a scale. Then plants were divided into different parts: roots, stem, leaves, and petioles. The fresh mass was recorded using an electronic balance. Then plant parts were exposed to 105°C for 1 h and dried to constant mass at 75°C to determine the biomass.

Chlorophyll (Chl) content and leaf relative water content: The Chl content was measured in samples from three latest fully expanded trifoliate leaves at the V5 stage from each treatment using *SPAD 502* (*Minolta*, Japan). For relative water content, three most recently expanded trifoliate leaves from each treatment were destructively sampled and their fresh mass was recorded. Fresh leaf samples were kept in 100 ml of distilled water for a period of 24 h and then their turgid mass was recorded. Subsequently, the leaves were dried in an oven at 70°C for 48 h and their dry mass was recorded. Relative water content (RWC) was determined using the following formula (Faijunnahar *et al*. 2017): RWC = [(FM – DM)/(TM – DM)] ×100, where FM = fresh mass; DM = dry mass; TM = turgid mass.

Leaf gas-exchange parameters: Three fully expanded trifoliate leaves of soybean plants at the V5 stage from each treatment were selected and their photosynthetic characteristics [net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (E)] were measured using a *Li-6400* portable photosynthesis system (*LI-COR Inc.*, Lincoln, NE, USA) under steady light intensity from 09:00–11:00 h. The equipment settings used were: PAR = 1,000 μmol(photon) m^{-2} s^{-1}, stomatal ratio = 0.5, flow = 500 μmol mol^{-1}, and reference CO_2 concentration = 400 μmol mol^{-1}. The aperture size of the instrument was 6 cm^2 and the leaf temperature was 26°C.

Chl fluorescence: A previously published method was followed by using *FluorImager* software (*Technologica, version 2.2.2.2*) (Hussain *et al.* 2019b). Three latest fully expanded trifoliate leaf samples from each treatment were taken at the V5 stage and immediately preserved in plastic bags and placed in an icebox to prevent direct light. Then, by using the above-mentioned software, samples were passed through the fluorescence analyzing device. Images of the minimum Chl fluorescence yield (F_0) in the dark-adapted state were captured using low-frequency light pulses (1 Hz). The maximum fluorescence (F_m) was determined by applying a blue saturation pulse (10 Hz). The maximum quantum yield of the PSII photochemistry (F_v/F_m ratio) was determined, and images were captured. Actinic illumination [1,000 μmol(photon) m^{-2} s^{-1}] was the same for all the samples. Photochemical efficiency of PSII, photochemical quenching (q_P), and electron transport rate [ETR; ETR= Φ_{PSII} × PAR × 0.5 × α, where PAR = 1,000; α = 0.85] were examined by placing leaves for 20 min under light and dark conditions.

Rubisco activated enzyme: To determine the Rubisco activated enzyme, a previously described method was used (Hussain *et al.* 2019b). The Rubisco ELISA kit (96 micropores) was purchased from *Shanghai Fu Life Industry Co*., *Ltd*. (Shanghai, China). The double antibody sandwich method was used to determine the content of plant Rubisco activase. One g of frozen leaf samples of each treatment was ground using 2 ml of 50 mmol L^{-1} phosphate buffer solution (pH 7.8) with the help of mortar and pestle in an icebox. The homogenate was then centrifuged at 4°C for 15 min at 7,000 rcf, the micropore plate encapsulated the Rubisco activase antibody to form a solid phase antibody. This was added to the micropore of the monoclonal antibody. Then a phosphate buffer solution (40 μl) was added first as a buffer solution in the micropore plate, then 10 ml of sample solution was added. The micropore plate was then sealed by a plastic film and incubated at 37°C for 30 min. The incubation was repeated over five times. The 3,3'5,5'-tetramethylbenzidine was transferred under the catalysis of the horseradish peroxidase enzyme, which first turned blue and finally to a yellow color under the action of an acid. The stop solution was added, and absorbance was measured within 15 min at 450 nm by an enzyme marker. Then, a standard curve was drawn and Rubisco activity was expressed as U g^{-1}.

Yield component analysis: To evaluate the impact of drought and shade stress on the yield of tested plants, seeds were harvested manually for both cultivars at the maturity stage and then air-dried. Yield components, such as seed yield per plant [g], 100-seed mass [g], the number of grains per plant, the number of pods per plant, and the number of infertile pods per plant, were determined.

Statistical analysis: Factorial analysis of variance (*ANOVA*) test was performed to test the effect of different drought treatments on the parameters of soybean under a shade and normal light using the *Statistix 8.1* software. A significant difference between treatment means was evaluated using *Duncan*'s multiple range test (p<0.05).

Results

Morphological parameters: Fig. 1 shows the effect of shade and drought stress on soybean morphological parameters at the V5 stage. Shade significantly increased the plant height in both cultivars, the maximum height (96 cm) was observed in T1 treatment for C103 while the minimum value (31 cm) was recorded in T3 treatment for N12. The plant height decreased significantly as the drought level intensified. The stem, root, petiole, and leaf mass also decreased significantly under shade conditions in comparison to full light for both the cultivars, while the decrease was greater in C103 in comparison to N12. Similarly, the fresh mass for different plant parts decreased with the increasing severity of drought stress. The maximum values for the stem, root, petiole, and leaf mass (4.12, 5.49, 1.96, and 6.39 g, respectively) were recorded for T1 in N12 under full light while minimum values (0.93, 0.93, 0.56, and 1.96 g, respectively) were recorded for T3 in C103 under shade conditions.

Leaf area and relative water content: Leaf area declined significantly with increasing levels of drought stress under both light regimes in both cultivars. However, in comparison to full light, leaf area decreased significantly (by 10.9, 8.9, and 7.6% and by 11.6, 9.0, and 18.1% in T1, T2, and T3 in N12 and C103, respectively) under shade environment for all moisture treatments in both cultivars. The maximum value (43.09 cm^2) for the leaf area was recorded in T1 in N12 under full light while the minimum value (20.95 cm^2) was recorded in T3 in C103 under shade conditions. The interaction of the light environment and moisture treatments was found significant. Decreasing

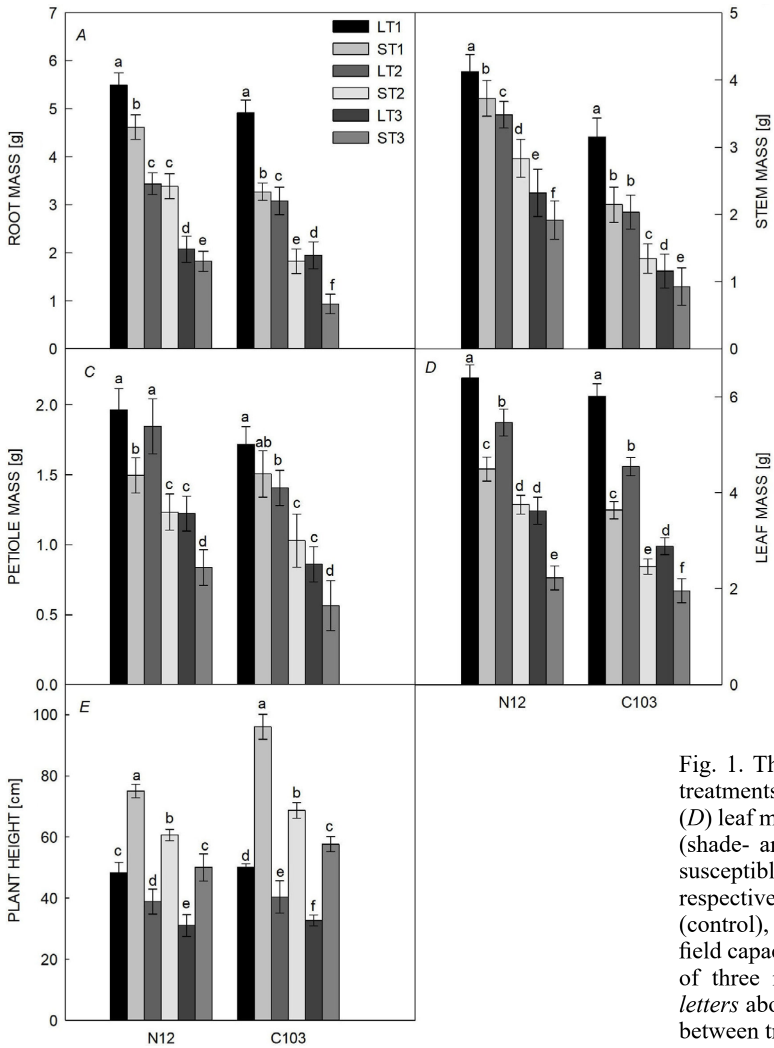

Fig. 1. The effect of different light regimes and drought stress treatments on (*A*) root mass, (*B*) stem mass, (*C*) petiole mass, (*D*) leaf mass, and (*E*) plant height of two soybean cultivars, N12 (shade- and drought-resistant) and C103 (shade- and drought-susceptible), respectively. L and S refer to full light and shade, respectively. T1, T2, and T3 refer to 75 ± 2% field capacity (control), 55 ± 2% field capacity (moderate drought), and 35 ± 2% field capacity (severe drought), respectively. Values are the mean of three replicates. Bars indicate ± SD. *Different lowercase letters* above the bars represent a significant difference ($P<0.05$) between treatments.

soil moisture content also led to a significant reduction in relative water content (RWC) under both light regimes in both cultivars. The overall RWC was significantly higher under shade conditions as compared to full light for both cultivars (Fig. 2) with maximum (76.6%) and minimum (63.0%) values noticed in T1 for N12 under shade environment and T3 in C103 under full light, respectively. The results suggested that the shade conditions helped in mitigating the drastic effects of drought stress in both soybean cultivars.

Leaf gas-exchange parameters and SPAD value: The leaf gas-exchange parameters and SPAD values were also highly influenced by drought stress under both light regimes in both soybean cultivars (Fig. 3). Increasing the severity of drought stress significantly decreased the photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and SPAD value in the two cultivars, the decrease was greater in C103 in comparison to N12. The shade conditions, however, helped ameliorate the negative effects of drought stress in both cultivars. P_N increased significantly in T2 and T3 by 16 and 21.3% in N12 and C103, respectively, under shade conditions in comparison to full light. Initially, the E, g_s, and SPAD value in T1 under shade conditions decreased significantly by 11.1, 7.6, 7.5, and 15.6, 15.7, 7.3% in N12 and C103, respectively, but a significant increase was observed in E (15.6 and 16.8%) and g_s (18.5 and 67.6%) for T2 and T3 in N12, respectively. Similarly, E (16.8%) and g_s (138.8%) improved significantly for T3 in C103, respectively. The SPAD value also improved significantly for T2 (4.7%) in N12 and T3 (8.2%) in C103, respectively. The maximum values for P_N [12.76 μmol(CO_2) m^{-2} s^{-1}], E [3.56 μmol(H_2O) m^{-2} s^{-1}], g_s (0.17 mmol m^{-2} s^{-1}), and SPAD (43.33) were recorded in treatment LT1 of N12 while minimum values for P_N [3.7 μmol(CO_2) m^{-2} s^{-1}], E [1.1 μmol(H_2O) m^{-2} s^{-1}], g_s (0.02 mmol m^{-2} s^{-1}), and SPAD (29.59) were recorded in treatment LT3 of C103. Our results demonstrated that under increasing drought stress conditions, the shade environment led to better photosynthetic performance (*i.e.*, under treatment T2 in N12 and treatment T3 in C103) by improving E, g_s, and SPAD values.

Chl fluorescence parameters: Increasing the severity of moisture stress significantly reduced the efficacy of photochemical machinery of both soybean cultivars

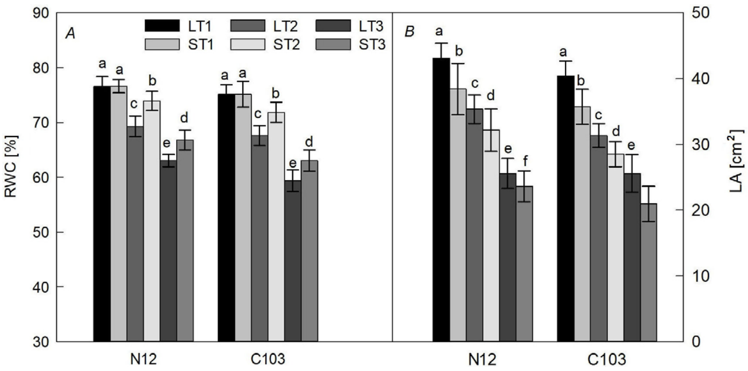

Fig. 2. The effect of different light regimes and drought stress treatments on (*A*) relative water content RWC and (*B*) leaf area (LA) of two soybean cultivars, N12 (shade- and drought-resistant) and C103 (shade- and drought-susceptible), respectively. L and S refer to full light and shade, respectively. T1, T2, and T3 refer to 75 ± 2% field capacity (control), 55 ± 2% field capacity (moderate drought), and 35 ± 2% field capacity (severe drought), respectively. Values are the mean of three replicates. Bars indicate ± SD. *Different lowercase letters* above the bars represent a significant difference ($P<0.05$) between treatments.

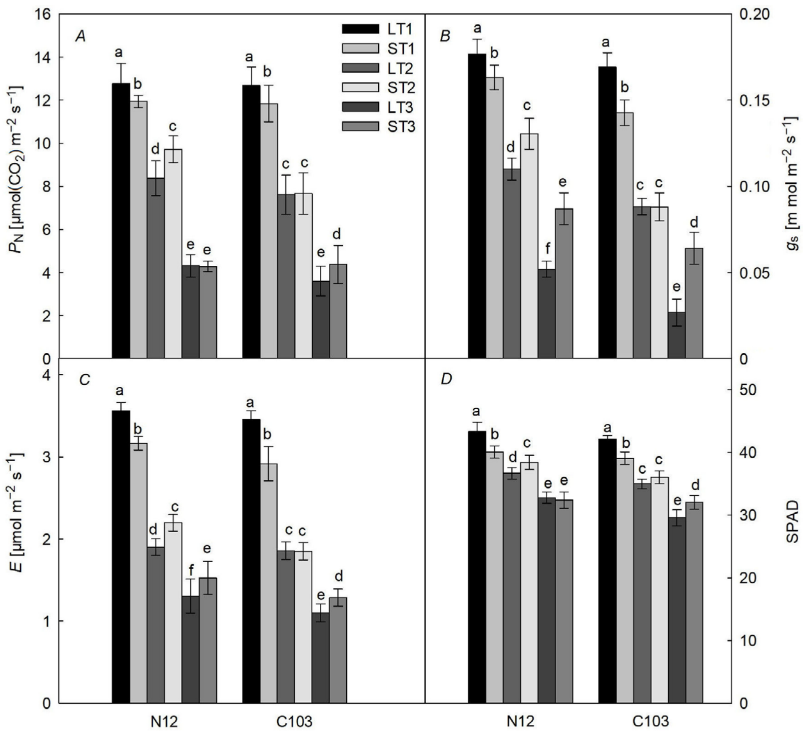

Fig. 3. The effect of different light regimes and drought stress treatments on (*A*) net photosynthesis (P_N), (*B*) stomatal conductance (g_s), (*C*) transpiration rate (*E*), and (*D*) SPAD value of two soybean cultivars, N12 (shade- and drought-resistant) and C103 (shade- and drought-susceptible), respectively. L and S refer to full light and shade, respectively. T1, T2, and T3 refer to 75 ± 2% field capacity (control), 55 ± 2% field capacity (moderate drought), and 35 ± 2% field capacity (severe drought), respectively. Values are the mean of three replicates. Bars indicate ± SD. *Different lowercase letters* above the bars represent a significant difference ($P<0.05$) between treatments.

indicating that changes in photosynthetic rate under increasing moisture stress were directly associated with the changes in Chl fluorescence parameters (Fig. 4). The maximum quantum yield (F_v/F_m), effective quantum yield of PSII (Φ_{PSII}), photochemical quenching (q_P), and electron transport rate (ETR) decreased significantly with increasing severity of drought stress under both light regimes in both cultivars, however, the decrease was significantly higher in C103 in comparison to N12. In comparison to full light, the shade environment significantly improved F_v/F_m, Φ_{PSII}, q_P, and ETR in T2 by 2.5, 3.5, 2.0, and 5.2% in N12 and in T3 by 3, 3.7, 4.7, and 3.7% in C103, respectively. The maximum values of F_v/F_m (0.804), Φ_{PSII} (0.243), q_P (0.450), and ETR (103.3) were observed in N12 in T1 under full light while minimum values of F_v/F_m (0.707), Φ_{PSII} (0.201), q_P (0.390), and ETR (85.595) were found in C103 in T3 under normal light conditions, respectively. The interaction of light environment and drought stress was found to be highly significant for all the recorded photochemical parameters.

Rubisco activity: The Rubisco activity (RA) of both soybean cultivars decreased significantly as the drought stress intensified, the decrease being significantly higher in C103 in comparison to N12 (Fig. 5). The maximum (0.281 U g^{-1}) and minimum (0.173 U g^{-1}) values of RA were recorded in T1 and T3 in N12 and C103, respectively, under full light conditions. In comparison to full light, the shade environment played a positive role by significantly improving the Rubisco activity in treatment T2 in N12 and treatment T3 in C103, respectively. The RA increased by 6.8 and 3.7% and 0.5 and 6.2% in T2 and T3 in N12 and C103, respectively, under the shade environment. Overall, the interactive effect of light environment and the drought stress on RA was found to be highly significant.

Yield parameters: Increasing the severity of drought stress resulted in a significant reduction of the seed yield per plant, 100-seed mass, number of grains per plant, and the number of pods per plant in both cultivars (Fig. 6). However, shade significantly enhanced the seed yield,

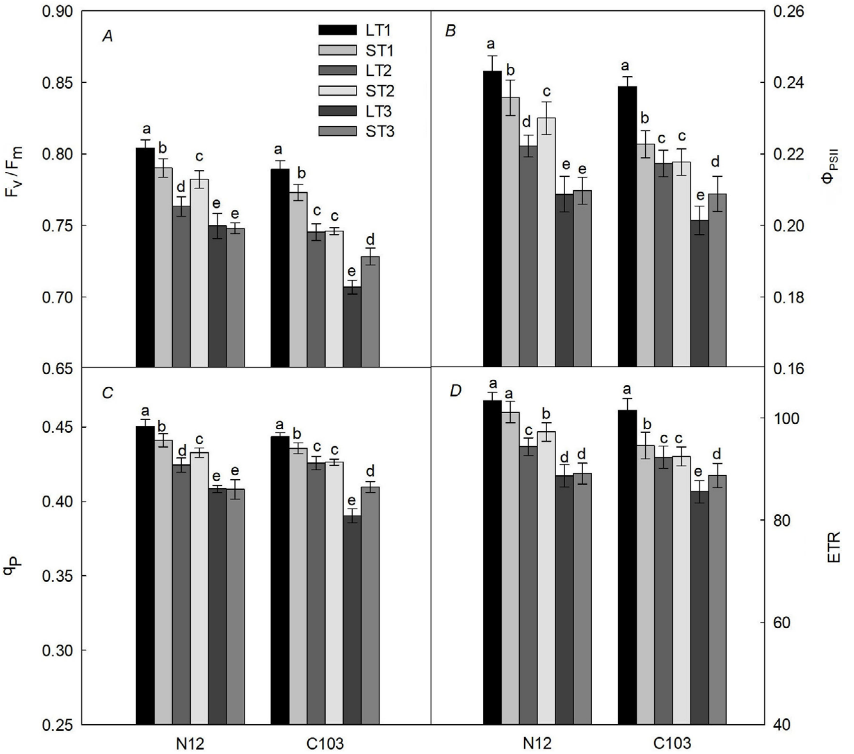

Fig. 4. The effect of different light regimes and drought stress treatments on (*A*) maximum quantum yield (F_v/F_m), (*B*) effective quantum yield of PSII (Φ_{PSII}), (*C*) photochemical quenching (q_P), and (*D*) electron transport rate (ETR) of two soybean cultivars, N12 (shade- and drought-resistant) and C103 (shade- and drought-susceptible), respectively. L and S refer to full light and shade, respectively. T1, T2, and T3 refer to 75 ± 2% field capacity (control), 55 ± 2% field capacity (moderate drought), and 35 ± 2% field capacity (severe drought), respectively. Values are the mean of three replicates. Bars indicate ± SD. *Different lowercase letters* above the bars represent a significant difference (P<0.05) between treatments.

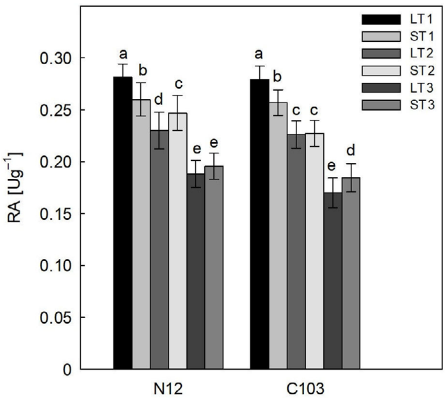

Fig. 5. The effect of different light regimes and drought stress treatments on Rubisco activity (RA) of two soybean cultivars, N12 (shade- and drought-resistant) and C103 (shade- and drought-susceptible), respectively. L and S refer to full light and shade, respectively. T1, T2, and T3 refer to 75 ± 2% field capacity (control), 55 ± 2% field capacity (moderate drought), and 35 ± 2% field capacity (severe drought), respectively. Values are the mean of three replicates. Bars indicate ± SD. *Different lowercase letters* above the bars represent a significant difference (P<0.05) between treatments.

100-seed mass, number of grains per plant, number of pods per plant in T2 and T3 by 6.9, 4.8, 10.6, 14.8% and by 15.1, 6.4, 9.3, 10.5% in N12 and C103, respectively. The maximum values for seed yield per plant (24.11 g), 100-seed mass (17.63 g), number of grains per plant (147.6), and number of pods per plant (53.6) were recorded in treatment LT1 of N12 while minimum values for seed yield per plant (11 g), 100-seed mass (14.02 g), number of grains per plant (92.3), and number of pods per plant (25.3) were recorded in treatment LT3 of C103. The number of infertile pods increased significantly with increasing levels of drought stress under full light conditions in both cultivars. However, shade resulted in a significant reduction in the number of infertile pods in treatment T2 of N12 (by 33.0%) and in T3 of C103 (by 10.3%), respectively. The maximum number of infertile pods (14.3) were recorded in treatment LT3 of C103 while minimum value (3.0) was recorded in treatment LT1 of N12, respectively.

Discussion

In the present study, both shade and drought stress limited the growth of soybean cultivars. Increasing the severity of drought stress resulted in a reduction of biomass production in both soybean cultivars which is also supported by earlier research (Du *et al*. 2020). The plant height decreased under drought conditions whereas shade led to a slender stem and increased plant height with the increase being greater in C103 in comparison to N12 which shows that the tolerant variety exhibited less shade avoidance and was not prone to lodging (Wu *et al*. 2017, Hussain *et al*. 2020b).

Leaf area is considered to be an important factor that affects the light interception in plants and biomass production (Yao *et al*. 2016). Reduction in leaf area under shade and drought was observed as a strategy of plants to reduce water loss under conditions of water scarcity and is consistent with earlier research (Dong *et al*. 2019). This reduction probably leads to the reduction in the soybean biomass accumulation which has been confirmed

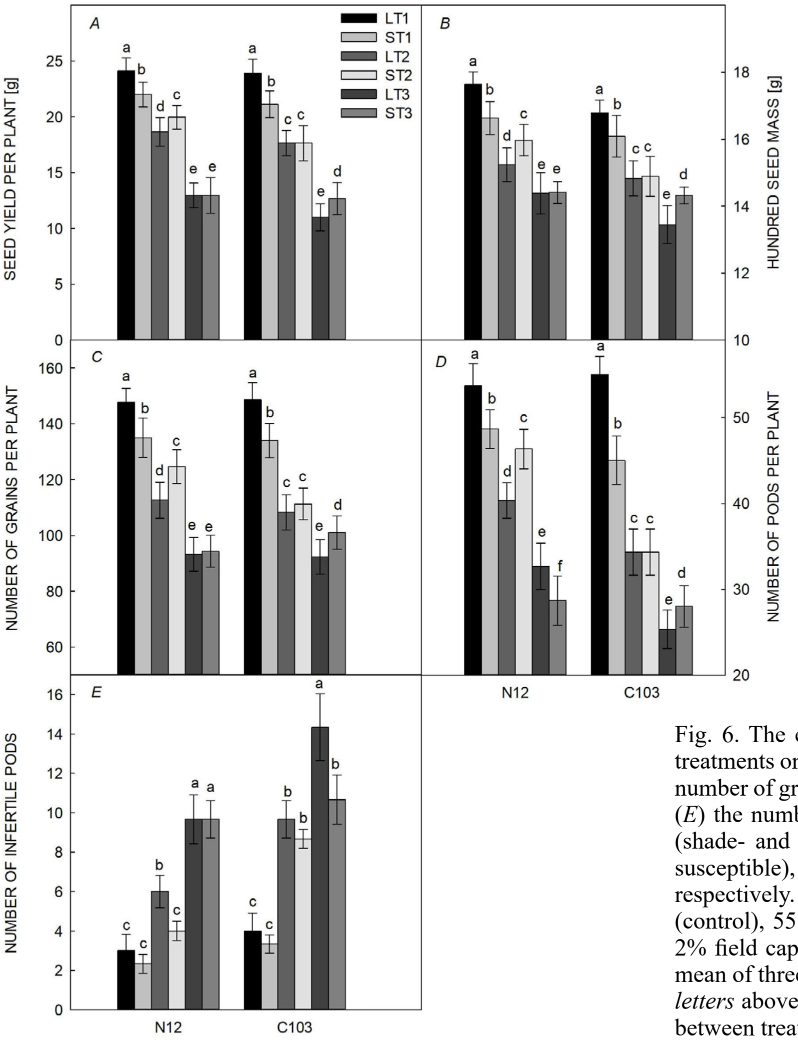

Fig. 6. The effect of different light regimes and drought stress treatments on (*A*) seed yield per plant, (*B*) 100-seed mass, (*C*) the number of grains per plant, (*D*) the number of pods per plant, and (*E*) the number of infertile pods of two soybean cultivars, N12 (shade- and drought-resistant) and C103 (shade- and drought-susceptible), respectively. L and S refer to full light and shade, respectively. T1, T2, and T3 refer to 75 ± 2% field capacity (control), 55 ± 2% field capacity (moderate drought), and 35 ± 2% field capacity (severe drought), respectively. Values are the mean of three replicates. Bars indicate ± SD. *Different lowercase letters* above the bars represent a significant difference (P<0.05) between treatments.

earlier by Su *et al*. (2014) and Wang *et al*. (2014). The relative water content (RWC) was also regressively reduced with increasing drought stress while higher RWC values observed under shade may be attributed to higher relative humidity and lower temperature under a shaded environment. The shaded plants need less water in comparison to those grown under full light as they can conserve water and require less water for transpiration (Holmgren 2000, Li *et al.* 2011).

Plants alter their photosynthetic characteristics to acclimate to various environmental conditions (Hussain *et al*. 2020c). Drought stress is reported to negatively affect the leaf gas-exchange parameters under full light as well as shade conditions (Chaves *et al*. 2009, Duan *et al*. 2009, Li *et al*. 2011). Our study was also consistent with these findings, the decrease in P_N was attributed to the decrease in g_s and E. The decrease was greater for C103 in comparison to N12 which proves the high drought resistance of N12 (Iqbal *et al*. 2019). However, in comparison to full light, under shade conditions, P_N increased significantly which depicts a positive role of shade under drought conditions and supports the facilitation hypothesis (Holmgren 2000, Quero *et al*. 2006). Moreover, the reduction ratio for all the measured gas-exchange parameters was lesser in the shade environment as compared to full light. Besides, the SPAD value also decreased in both cultivars as the level of drought intensified (Gunes *et al*. 2008) but the reduction was greater under full light in comparison to the shade environment. Also, the SPAD values for N12 were greater in comparison to C103 which probably contributed to the better photosynthetic performance of the former cultivar.

In our present study, as the severity of drought stress increased, F_v/F_m, q_P, Φ_{PSII}, and ETR decreased significantly under both light regimes which is consistent with the earlier reported research (Hussain *et al*. 2019c, Iqbal *et al.* 2019). The decrease was recorded to be greater in plants grown under full light as compared to those grown under shade conditions. Moreover, the decrease in Chl fluorescence parameters was greater in C103 in comparison to N12 which suggests that the structural integrity of PSII of N12 was not damaged by stress conditions. N12 proved to be a resistant cultivar with better photosynthetic performance and agricultural productivity.

A rapid decrease in RA is a prominent plant response against drought stress (Parry *et al*. 2002). In our study, RA decreased under both light regimes as the severity of drought stress increased. This is consistent with earlier reported literature (Majumdar *et al*. 1991, Bota *et al*. 2004). The reduced RA contributed to the reduction of photosynthetic performance as Rubisco plays an important role in the biochemical limitation of photosynthesis in plants under water deficit (Perdomo *et al*. 2017). Interestingly, in comparison to full light, RA increased under shade conditions, which is why comparatively better P_N values were obtained under shade environment than under full light conditions.

Drought and shade stresses are the prime abiotic constraints that substantially affect the seed yield by reducing the pod and seed number, eventually affecting the commercial trait '100-seed mass'. In our study, the seed yield of both cultivars decreased significantly as the severity of drought stress increased. This is in line with the earlier research (Desclaux *et al*. 2000, Liu *et al*. 2003, Stolf-Moreira *et al*. 2010, Iqbal *et al*. 2018). However, the results of our study demonstrated a significantly higher seed yield of N12 (under moderate drought stress) and C103 (under severe drought stress) under shade conditions as compared to full light. This indicates a better performance of soybean plants exposed to drought stress under shade which shows that a moderate shade can help soybean to mitigate the drought stress. Overall, N12 showed better yield production in comparison to C103 which proves its resistance to shade and drought stress.

Conclusion: Our research demonstrated that increasing the level of drought stress resulted in the poor photosynthetic performance of soybean cultivars due to reduction in leaf area, relative water content, leaf gas-exchange parameters (*i.e*., P_N, E, and g_s), chlorophyll fluorescence parameters (*i.e*., F_v/F_m, Φ_{PSII}, q_P, and ETR), chlorophyll content, and Rubisco activity which ultimately led to yield reduction. In conclusion, with the appropriate selection of cultivars, a moderate shade can help to optimize yield and improve the performance of soybean exposed to drought stress in maize–soybean relay intercropping system. However, further research considering the effect of different shade levels on soybean performance under drought conditions is required under field conditions. It is obvious that under field conditions, in the presence of maize, the effects of inter- and intraspecific competition can play an important role in altering the growth, photosynthesis, and drought tolerance of soybean. Therefore, further research in this direction, when considering the above-mentioned facts, can provide a better insight into the role of shade in the alleviation of drought stress in soybean under maize–soybean relay intercropping system.

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