DOI: 10.32615/ps.2019.036 PHOTOSYNTHETICA 57 (1): 248-257, 2019

Interactive effects of temperature and phosphorus nutrition on soybean: leaf photosynthesis, chlorophyll fluorescence, and nutrient efficiency

S.K. SINGH[*,**,+], V.R. REDDY[*], D.H. FLEISHER[*], and D.J. TIMLIN[*]

Adaptive Cropping Systems Laboratory, USDA-ARS, Beltsville, MD, USA[*]
School of Environmental and Forest Sciences, University of Washington, WA, USA[**]

Abstract

An experiment was conducted to assess interactive effects of temperature (22, 26, 30, and 34°C daily mean T) and phosphorus (P) fertilization (sufficient, 0.5 mM, and deficient, 0.08 mM P) on soybean physiological traits. The P deficiency decreased leaf P concentration over approximately 50% across temperature regimes. However, a marked decrease in physiological traits under P deficiency was primarily observed below and at optimum temperature (26°C) but not at warmer temperatures. This resulted in a significant P × T interaction for parameters such as net photosynthetic rate (P_N), stomatal conductance, quantum yield of PSII (Φ_{PSII}), and SPAD value. A combination of photo-biochemical parameters (*e.g.*, Φ_{PSII}, carboxylation capacity, SPAD value), improved CO_2 diffusion processes due to unaffected or reduced mesophyll or stomatal limitation, and higher tissue P utilization efficiency appeared to overcome limitations to P_N imposed by P deficiency at warmer temperatures.

Additional key words: chlorophyll fluorescence; compensation; coregulation; nutrient utilization efficiency; optimum temperature.

Introduction

Soybean [*Glycine max* (L.) Merr.] is an important row crop grown worldwide as a source of protein and vegetable oil (Hartman *et al*. 2011). Plant stresses such as nonoptimal (below and above the optimum) temperatures and P deficiency adversely affects photosynthetic processes, growth, and productivity of crops including soybean (Cure *et al.* 1988, Israel and Rufty 1988, Koti *et al.* 2007, Ruiz-Vera *et al.* 2013, Xu *et al.* 2016). High temperature reduces soybean net photosynthesis but increases stomatal conductance (g_s) and transpiration, whereas P deficiency reduces both the net photosynthesis and g_s (Ruiz-Vera *et al.* 2013, Singh and Reddy 2016, Xu *et al.* 2016). In addition, both the stress situations strongly affect chlorophyll fluorescence (CF) processes of the PSII reaction center of the chloroplast and cellular membrane integrity (Koti *et al.* 2007, Singh and Reddy 2015, Xu *et al.* 2016). Previous studies in soybean have shown that a temperature above the optimum often leads to the restricted diffusion of CO_2 inside the leaves by increasing the stomatal and mesophyll limitations to the photosynthesis (Bernacchi *et al.* 2002, Xu *et al.* 2016). However, an increased stomatal or mesophyll limitation to photosynthesis was not observed in the P-deficient soybean despite a decrease in stomatal and mesophyll conductance (Singh and Reddy 2016). Studies evaluating crop response to P fertilization below and above the optimum temperature are extremely limited and to our knowledge, unavailable in soybean (Suriyagoda *et al.* 2012).

P deficiency also affects the intrinsic nutrient utilization efficiency and dynamics of other nutrients such as nitrogen (N) inside the plants (Israel and Rufty 1988, Fleisher *et al.* 2012, Singh *et al.* 2014, Singh and Reddy 2015). An increased tissue N concentration and N/P ratio of plant organs including seeds in P-deficient soybean have been reported and was attributed to a greater N uptake and storage by plants (Singh *et al.* 2014). Moreover, P deficiency has been shown to increase plant tissue P-utilization efficiency for photosynthesis and biomass production under optimal temperature regime (Cure *et al.* 1988, Singh *et al.* 2014). However, effects of nonoptimal temperatures on the tissue P-utilization efficiency of soybean photosynthetic processes are unclear.

Temperature and precipitation are among the most important environmental drivers that control the crop's agroecological distribution and productivity. Both of these factors have been strongly affected by the alterations in the global climate, which are most likely caused by greenhouse gas emissions and land use changes (Stocker *et al.* 2013). The global mean surface air temperature is most likely to rise >1.5°C by the end of 21st century, and this will have

Received 5 April 2017, *accepted* 12 September 2018.
[+]Corresponding author; phone: +1301-504-6633, fax: +1301-504-5823, e-mail: shardendu.singh@ars.usda.gov, singhsk@uw.edu.
Abbreviations: Chl – chlorophyll; CMT – cell membrane thermostability; g_m – mesophyll conductance; g_s – stomatal conductance; NUE – nitrogen-utilization efficiency; P_N – net photosynthetic rate; PUE – phosphorus-utilization efficiency; SPAD value – an indicator of chlorophyll content, V_{Cmax} – maximal rate of carboxylation, Φ_{CO2} – quantum yield of CO_2 fixation; Φ_{PSII} – photochemical quantum yield of PSII.
Acknowledgments: Authors thank Emily Morris, Jackson Fisher, and Darryl Baxam for help in maintaining the growth chambers. Thanks also goes to the visitors form the Republic of Korea Seunghwan Wi, Agricultural Institute of Climate Change and Sang Wangyu, National Institute of Crop Science for assisting in the measurements.

strong impacts on the productivity of most crops including soybean (Lobell and Asner 2003, Stocker *et al.* 2013). In addition, P deficiency limits crop production worldwide and its limited availability, as a natural resource, is of a global concern (Cordell *et al.* 2009). Therefore, the availability of plant nutrients will become increasingly important due to the depletion from agricultural lands over multiple years of cultivation, and higher crop nutrient demand due to increased productivity using hybrid cultivars and by the projected rise in atmospheric CO_2 concentration (Rogers *et al.* 1993, Römheld and Kirkby 2010).

Plant photosynthesis contributes to much of plant biomass accumulation and is highly sensitive to levels of P fertilization and temperature regimes (Taub 2010, Singh and Reddy 2015, Xu *et al.* 2016). P is an essential part of the cellular membranes, nucleic acid and is directly involved in the carbohydrate metabolism (Marschner 1995, Warren 2011, Singh and Reddy 2015). Most of the prior investigations on crop response to nonoptimal temperatures have often been done under adequate nutrient fertilization (Sinclair 1992). However, under natural growing conditions, crops such as soybean are simultaneously exposed to nonoptimal temperature and nutrient-limited conditions (Sinclair 1992, Mittler 2006). Therefore, it is imperative to understand the interactive effects of P and T on soybean physiology.

Soybean growth and physiological processes strongly correlate with the foliar P concentrations or with the growing temperatures (Cure *et al.* 1988, Campbell *et al.* 1990, Singh *et al.* 2014, Singh and Reddy 2015). Sinclair (1992) suggested that effects of environmental factors on plant productivity might be minimized under the nutrient-limited conditions. For instance, studies have shown that soybean P_N response to environmental factors such as elevated CO_2 concentration closely depends on the P fertilization of plants (Cure *et al.* 1988, Singh *et al.* 2014, Singh and Reddy 2016). Furthermore, P fertilizer application partly mitigated the adverse effects of drought in soybean grown under P deficiency (Jin *et al.* 2006), whereas N fertilization did not alleviate the negative impacts of heat stress on wheat (*Triticum aestivum* L.) (Mitchell *et al.* 1993). Thus, plant response to environmental factors appears to vary depending on the unique combination of stresses. The nature of plant physiological alterations and their magnitude and direction due to P × T interaction is yet to be investigated. Since soybean growth and physiological processes are highly related to the P fertilization, we hypothesize that their response to P nutrition will vary when grown under different temperature regimes. The objective of this study was to evaluate the impacts of P × T interaction on soybean photosynthesis and chlorophyll (Chl) fluorescence traits, nutrient-utilization efficiency, cell membrane thermostability, and the dynamics of leaf P and N concentration.

Materials and methods

Experimental conditions and plant culture: An experiment was conducted outdoors in the sunlit Soil-Plant-Atmosphere-Research (SPAR) chambers at the USDA-ARS facility in Beltsville, MD, USA. Each SPAR chamber consists of a steel soilbin (1 m deep, 2 m long, 0.5 m wide) for plant root growth sealed to a Plexiglas chamber (2.5 m tall, 2.2 m long, 1.4 m wide) to accommodate aerial plant parts and atmospheric conditions, a heating and cooling system, and an environmental monitoring and control system. Plexiglas transmits >90% of the ambient solar radiation inside the SPAR chambers (Kim *et al.* 2007). The details of the SPAR chambers and methods of operation and monitoring have been described previously (Fleisher *et al.* 2009, Timlin *et al.* 2017). In brief, the temperature is controlled by cooling and heating of the air inside chambers. Chilled ethylene glycol is supplied to the cooling system *via* solenoid valves depending on the cooling requirements. Electrical resistance heaters provide pulses of heat, as needed, to fine-tune the air temperature. The air passes over the cooling coil and heating elements through the top portion inside the chamber with a sufficient velocity to cause leaf flutter (2.5 m s^{-1}) and returns to the air-handling unit just above the soil level. Each chamber is equipped with an infrared gas analyzer (*LI-6262*, *LI-COR Inc*., NE, Lincoln, USA) and gas mass flow controller (*FM-766*, *Omega Engineering Inc*., Norwalk, CT, USA) to measure and control CO_2 inside the SPAR chambers. The continuous monitoring and control of all-important environmental variables in each chamber are done by a dedicated microcomputer workstation using a custom program (Baker *et al.* 2004, Fleisher *et al.* 2009).

Soybean [*Glycine max* (L.) Merr., cv. NC-Roy) was planted in nine rows (20 cm apart, five plants/row) in the soilbin of eight SPAR chambers filled with the mixture of 75% sand and 25% vermiculite on 17 June 2015. After emergence (*i.e.*, six days after planting), the treatments were initiated by setting each SPAR chamber to one of the four day/night temperatures (T) at below optimum (24/18°C), optimum (28/22°C, OT), and two warmer than OT (32/26°C and 36/30°C), and one of the two concentrations of phosphorus (P) nutrition at 0.5 mM (sufficient) and 0.08 mM (deficient). A modified Hoagland's nutrient solution was supplied as the fertigation (Hewitt 1952) 4–6 times per day in excess of daily water demand. Water drained through outlets at the bottom of each soilbin. The daytime temperature was initiated at sunrise and returned to the nighttime temperature one hour after sunset resulting in 16-h/8-h day/night thermo-period. The seasonal average daily mean air temperature inside the SPAR chambers was almost the same as the treatment set point at 22 ± 0.13°C, 26.1 ± 0.12°C, 29.9 ± 0.14°C, and 33.8 ± 0.12°C for the below OT, OT, and warmer temperature treatments, respectively, across the P nutrition. The SPAR chamber CO_2 was maintained at 420 ± 13 μmol mol^{-1} during the daytime and the relative humidity varied between 46 and 68% during the experiment. A destructive plant harvest was conducted at 29 d after planting to avoid plant competition by removing two alternate plants form the middle of all rows for all chambers. Thus, after this date, there were nine rows with three plants each (27 plants m^{-2}) in a given treatment during the measurements of the physiological traits.

Photosynthesis and Chl fluorescence: The photosyn-

thesis parameters and Chl fluorescence (CF) were measured simultaneously 36–37 d after planting on the uppermost fully expanded soybean leaves between 9:00 and 13:00 h from 3–4 plants in each treatment using a portable photosynthesis system (*LI-COR-6400XT*, *LI-COR Inc*., Lincoln, NE, USA) equipped with an integrated fluorescence chamber head (*LI-COR 6400-40* leaf chamber fluorometer). The photosynthesis was measured at the PAR of 1,500 µmol(photon) m^{-2} s^{-1}. The *LI-6400XT* cuvette leaf temperature was set to match the treatment daytime temperature, the CO_2 concentration was controlled at 400 µmol mol^{-1}, and the relative humidity varied between 45 and 60%. The P_N and the steady-state CF (F_s) measurements were taken when a steady state (around 4–6 min) was obtained. The maximal fluorescence (F_m') was measured using Multiphase Flash Protocol (MPF) by setting the ramp to 20%, phases 1 to 3 varied between 250 and 300 ms, and the flash intensity was set to nine that yielded the flash between 7,700 and 8,100 µmol(photon) m^{-2} s^{-1} among the measurements. These settings were obtained based on pretests the authors conducted on a representative soybean leaf across treatments. In the light-adapted leaves, the photochemical quantum yield of PSII (Φ_{PSII}) was determined following Maxwell and Johnson (2000) using the equation $\Phi_{PSII} = (F_m' - F_s)/F_m'$. These parameters along with stomatal conductance to water vapor (g_s) and CO_2 (g_{sCO2}), substomatal CO_2 concentration (C_i), and quantum yield of CO_2 assimilation (Φ_{CO2}) were computed using the instrument software (*LI-COR 6400XT Instruction Manual, version 6*).

Mesophyll conductance, maximal carboxylation rate, and photosynthetic limitations: The mesophyll conductance (g_m) and the maximum carboxylation rate (V_{Cmax}) were determined 38 d after planting according to Bunce (2009) and described by Singh and Reddy (2016). This method is based on the oxygen sensitivity of the Rubisco in the chloroplast. The g_m and V_{Cmax} were determined by measuring the response of photosynthesis to two different O_2 concentrations (ambient 21% and low 2%) under the strictly Rubisco-limited state as described by Singh and Reddy (2016). The g_m and V_{Cmax} were estimated iteratively by *PROC NLIN* procedure in *SAS* using a statistical analysis program given by Singh and Reddy (2016) where the day-time leaf respiration rate was set to 2.0 µmol(CO_2) m^{-2} s^{-1} based on the previous report indicating the averaged day respiration value near this level in the same cultivars and at the similar range of temperature treatments (Xu *et al.* 2016). The input coefficients including the Michaelis-Menten constants of Rubisco for CO_2 (K_c) and O_2 (K_o), respectively, and the temperature corrected chloroplast-based CO_2-compensation point (Γ*) were based on Sharkey *et al*. (2007). Briefly, the P_N at the two O_2 concentrations in the uppermost fully expanded leaves of 3–4 plants were measured similarly as described previously for the photosynthetic measurements except for the CO_2 concentration in the instrument leaf chamber that was set to 250 µmol mol^{-1}. The P_N was first measured at the ambient O_2 concentration followed by the 2% O_2 in the same leaf. To realize the low O_2, a gas cylinder containing 2% O_2 and 98% N_2 was connected to the *LI-COR 6400XT* inlet after passing through a rotometer to adjust the flow rate to a constant level. The sensitivity of the infrared gas analyzer to O_2 was corrected before recording the data by changing the O_2 concentration in the instrument software.

The estimation of stomatal (L_S), mesophyll (L_M), and biochemical (L_B) limitations to photosynthesis was conducted based on the relative changes in the P_N that is expressed in the terms of relative changes in the g_{sCO2}, g_m, and V_{Cmax} as proposed by Jones (1985) and extended by Grassi and Magnani (2005) using the equations:

$$L_S = \frac{(g_{tot}/g_{sCO2}) \times (\partial P_N/\partial C_c)}{g_{tot} + (\partial P_N/\partial C_c)} \times 100 \quad (1)$$

$$L_M = \frac{(g_{tot}/g_m) \times (\partial P_N/\partial C_c)}{g_{tot} + (\partial P_N/\partial C_c)} \times 100 \quad (2)$$

$$L_B = \frac{g_{tot}}{g_{tot} + (\partial P_N/\partial C_c)} \times 100 \quad (3)$$

where g_{tot} is the total conductance to CO_2 between leaf surface to carboxylation site ($1/g_{tot} = 1/g_{sCO2} + 1/g_m$). The C_c was calculated as $C_i - P_N/g_m$. The $\partial P_N/\partial C_c$ was calculated according to Sanglard *et al.* (2014) using the equation below:

$$\frac{\partial P_N}{\partial C_c} = \frac{V_{Cmax} \times (\Gamma^* + K_m)}{(C_c + K_m)^2} \quad (4)$$

where the K_m was calculated as K_c (1 + O/K_o), O is the ambient oxygen concentration.

SPAD value and cell membrane thermostability: The measurement of the SPAD value (an indicator of Chl content), was made on the same leaves, which were used for the photosynthetic measurements. The SPAD value was measured using a *SPAD 502 Plus Chlorophyll Meter* (*Spectrum Technologies Inc.*, Aurora, IL, USA) at three locations on each leaf and the averaged value was recorded.

The cell membrane thermostability (CMT) of the uppermost fully expanded leaves in 3–4 plants per treatment was assessed on 37 d after planting according to Martineau *et al*. (1979). In brief, four leaf disks (0.95 cm^{-2}) from each plant were prepared in two sets (C-set and T-set) of test tubes containing 10 ml of de-ionized water after washing thoroughly to remove the electrolytes adhering at the cut surface of disks. Test tubes were covered with the aluminum foil. Thereafter, the T-set received a 20-min water-bath treatment of 50°C while the C-set tubes were kept at ambient temperature during this time. An initial electrical conductivity reading of the solution of both sets (C1 and T1) was taken using a conductivity meter (*Corning Checkmate II*, *Corning Inc*., New York, NY, USA) after keeping them overnight in a cold room set at 18°C air temperature. Thereafter, to kill leaf tissue completely, both sets were autoclaved at 120°C and 0.15 MPa for 20 min, and the final electrical conductivity readings (C2 and T2) were made after tubes were cooled to the room temperature. The CMT was calculated using the equation based on Martineau *et al*. (1979) as:

$$\text{CMT (\%)} = \frac{1 - \left(\frac{T1}{T2}\right)}{1 - \left(\frac{C1}{C2}\right)} \times 100 \quad (5)$$

Tissue phosphorus and nitrogen concentration: The leaves were detached after the photosynthetic measurements to determine the individual leaf area, dry mass, and tissue phosphorus (P) and nitrogen (N) concentration. The leaves were dried in an oven at 70°C until constant mass and the dried material was ground using a *Wiley Mill* (*Wiley® Mill, Thomas Scientific,* Swedesboro, NJ, USA) to pass through a 1-mm screen. The P concentration of the dried material was determined at the Agriculture Diagnostic Laboratory, University of Arkansas, Fayetteville, AR, USA, using a standard procedure (Plank 1992). The N concentration of the dried material was determined by combustion using a *CHN-2000* (*Carbon Hydrogen Nitrogen-2000*, *LECO Corporation*, Saint Joseph, MI, USA). The P and N utilization efficiency (PUE and NUE, respectively) for P_N, V_{Cmax}, and Φ_{PSII} was estimated by dividing with the value of tissue P or N concentration based on leaf area (mg m^{-2}) of the same leaf.

Data analysis: A prior uniformity study using the same chambers indicated that no statistical differences were present for the plant growth between the SPAR chambers (Fleisher *et al.* 2009). Therefore, to test for the effect of treatments and their interaction on the measured traits, *PROC MIXED* procedure of the *SAS* (*SAS Enterprise Guide*, *4.2*, *SAS Institute Inc.,* Cary, NC, USA) with Kenward-Rogers (kr) adjustment of degrees of freedom was used for analysis of variance (*ANOVA*) using individual plants as pseudoreplicates. The *ANOVA* significance levels (*P*-values) are presented at the significance level $\alpha = 0.05$ is presented. The regression analysis was conducted using *PROC GLM* procedure of *SAS*.

Results

Tissue constituents and CMT: The leaf P concentration significantly declined 40–60% while the N concentration and N/P ratio increased 4–22% and 88–166%, respectively, in the P-deficient *vs*. P-sufficient leaves across T treatments (Fig. 1*A–C*). However, these traits along with the Chl content did not show a significant effect of T (Fig. 1*C*). There was significant P × T interaction for SPAD value and CMT (Fig. 1*D*,*E*). The P deficiency decreased (≈21%) the Chl content, particularly, below and at the OT (22 and 26°C) (Fig. 1*D*). The P deficiency increased CMT by 25.8% at 22°C, but it either did not affect at OT or decreased 16–18% at warmer T (Fig. 1*E*). Moreover, the warmer than OT decreased CMT, especially, in the P-deficient leaves.

Photosynthetic parameters: There was a significant P × T interaction for P_N and g_s (Fig. 2*A*,*B*). Relative to sufficient P nutrition, the P_N declined 47 and 36% under P deficiency at 22 and 26°C, respectively, but remained almost similar at the warmer T treatments (Fig. 2*A*). Relative to the OT, the P_N slightly declined by 5–9% at colder and warmer T under sufficient P, however, it was either 21% smaller at below OT or 43% greater at warmer than OT under the P-deficient condition (Fig. 2*A*). The g_s also declined 34–55% under P deficiency, especially, at and below the OT (Fig 2*B*). In contrast, g_s increased as temperature increased up to the two warmest treatments, particularly, in the P-deficient leaves (Fig. 2*B*). Under P deficiency, the V_{Cmax} decreased 36–50% at and below the OT but showed similar values at warmer T (Fig. 2*C*). The V_{Cmax} also increased with the T across both P nutrition (Fig. 2*C*). Under P deficiency, the g_m was 24–42% smaller across T treatments and showed the greatest value at the warmest T treatment (Fig. 2*D*).

Chl fluorescence parameters: There was a significant P × T interaction for Φ_{CO2} and Φ_{PSII} exhibiting a consistent decline by 33–48% under P deficiency at and below the OT but not at the warmer temperatures (Fig. 3*A–B*). These parameters also increased as the T increased, especially in the P-deficient leaves. The Φ_{PSII}/Φ_{CO2} was not affected by the P treatment but increased ≈18% at the warmest T *vs*. OT (Fig. 3*C*).

The stomatal, mesophyll, and biochemical limitations to P_N: A significant effect of T was observed for L_S and L_M (Fig. 4*A*,*B*). Relative to the OT, the L_S slightly declined by 11–15% at colder and warmer T under P deficiency, however, it was either 18–23% smaller at below OT and at 30°C (daily mean T) or 18% greater at the warmest T under sufficient P condition (Fig. 4*A*). Relative to the OT, the L_M was almost similar at the colder T but either increased by 45% at 30°C (daily mean T) or decreased by 18% at the warmest T, when averaged between P concentrations (Fig. 4*B*). The effects of treatments for L_B was not statistically significant (Fig. 4*C*).

Relationship of Chl content with leaf N, photosynthesis, and Chl fluorescence parameters: There were significant curvilinear regression relationships of SPAD value with the leaf N concentration, P_N, and Φ_{PSII} (Fig. 5). A single polynomial second order function across the treatments (P and T) best described their regression relationships for each parameter. The leaf N concentration tended to decrease while P_N, and Φ_{PSII} increased curvilinearly as the Chl content increased (Fig. 5).

Nutrient-utilization efficiency: The main effect of treatments (P and T) was significant for PUE and NUE of photosynthetic and Chl fluorescence parameters (Fig. 6). The PUE of P_N, V_{Cmax}, and Φ_{PSII} was greater under P deficiency across temperature regimes, except at the lowest temperature, with the highest increases (90–133%) observed under the warmer than OT (Fig. 6*A–C*). In contrast, the NUE of these parameters was smaller under P deficiency across temperatures with the greatest decrease (27–62%) found below or at the OT (Fig. 6*D–F*). Several of these parameters also had a significant P × T interaction exhibiting a distinct temperature response pattern between the two P nutrition. For example, the PUE of P_N, V_{Cmax}, and Φ_{PSII} almost consistently increased with temperature under P deficiency but varied under the sufficient P (Fig. 6*A–D*).

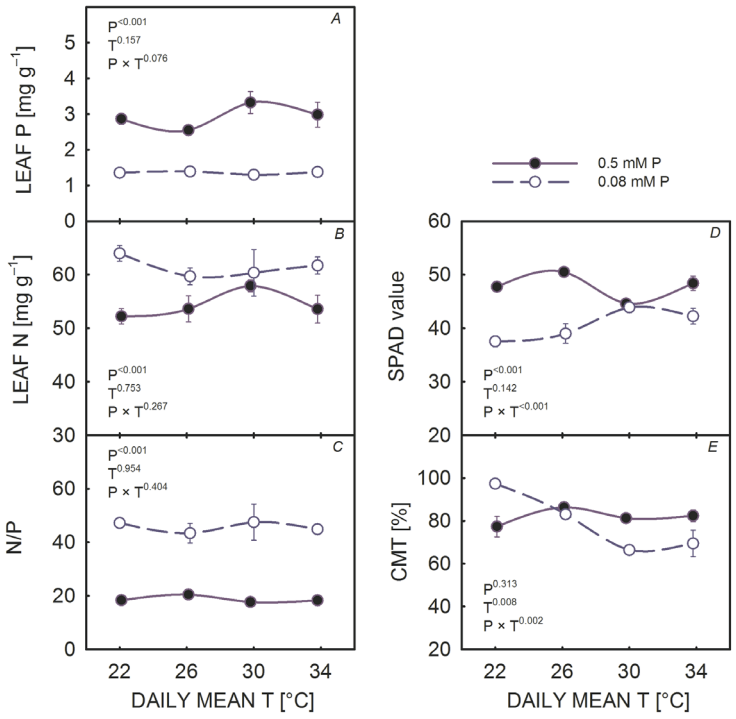

Fig. 1. Response of the tissue (*A*) phosphorus (P) and (*B*) nitrogen (N) concentrations, (*C*) N/P ratio, (*D*) SPAD value, and (*E*) cell membrane thermostability (CMT) to temperature (T) in the uppermost fully expanded leaves of soybean grown under two phosphorus (P) nutrition. Symbols represent the mean ± standard error (n = 3–4). Error bars smaller than the symbol are not visible. The significance level (P-value) of the analysis of variance for the effect of treatments and their interaction (P, T, P × T) is also shown.

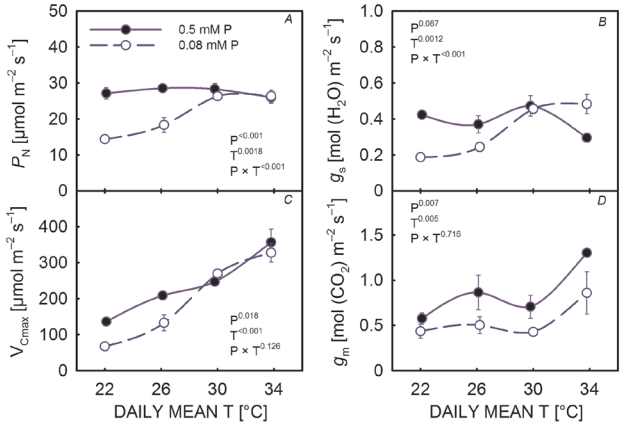

Fig. 2. Response of the (*A*) photosynthetic rate (P_N), (*B*) maximal rate of carboxylation (V_{Cmax}), (*C*), stomatal conductance (g_s), and (*D*) mesophyll conductance (g_m) to temperature (T) in the uppermost fully expanded leaves of soybean grown under two phosphorus (P) nutrition. Symbols represent the mean ± standard error (n = 3–4). Error bars smaller than the symbol are not visible. The significance level (P-value) of the analysis of variance for the effect of treatments and their interaction (P, T, P × T) is also shown.

A similar pattern was also observed for the NUE of P_N, and Φ_{PSII} (Fig. 6*D*,*F*).

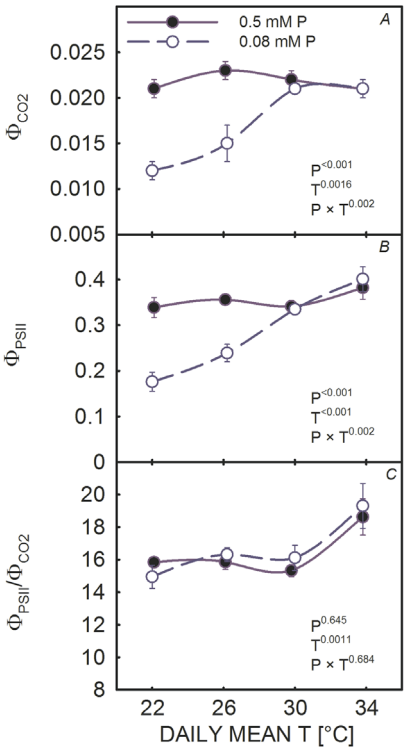

Fig. 3. Response of the (*A*) quantum yield of CO_2 fixation ($_{CO2}$), (*B*) photochemical quantum yield of PSII (Φ_{PSII}), and (*C*) Φ_{PSII}/Φ_{CO2} ratio to temperatures (T) in the uppermost fully expanded leaves of soybean grown under two phosphorus (P) nutrition. Symbols represent the mean ± standard error (n = 3–4). Error bars smaller than the symbol are not visible. The significance level (P-value) of the analysis of variance for the effect of treatments and their interaction (P, T, P × T) is also shown.

Discussion

In this study, the interactive impacts of temperature and P nutrition on soybean physiological traits were investigated. Results showed that P deficiency adversely affected soybean physiological traits primarily at and below the OT. However, under warmer than optimum

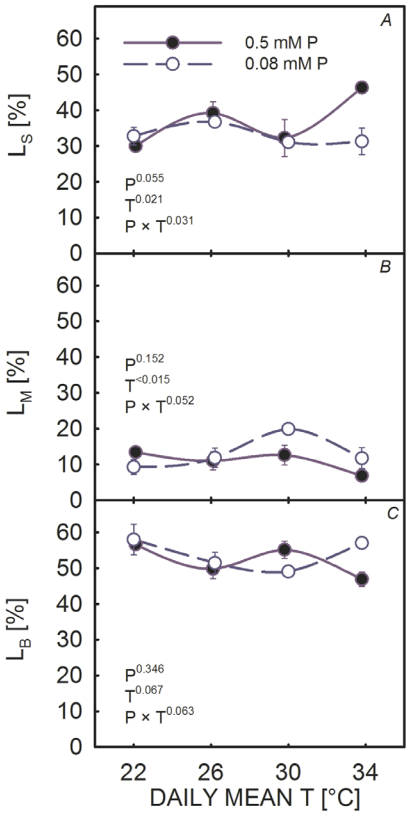

Fig. 4. Response of (*A*) stomatal (L_S), (*B*) mesophyll (L_M), and (*C*) biochemical (L_B) limitations to photosynthesis in the uppermost fully expanded leaves of soybean grown under two phosphorus (P) nutrition. Symbols represent the mean ± standard error (n = 3–4). Error bars smaller than the symbol are not visible. The significance level (P-value) of the analysis of variance for the effect of treatments and their interaction (P, T, P × T) is also shown.

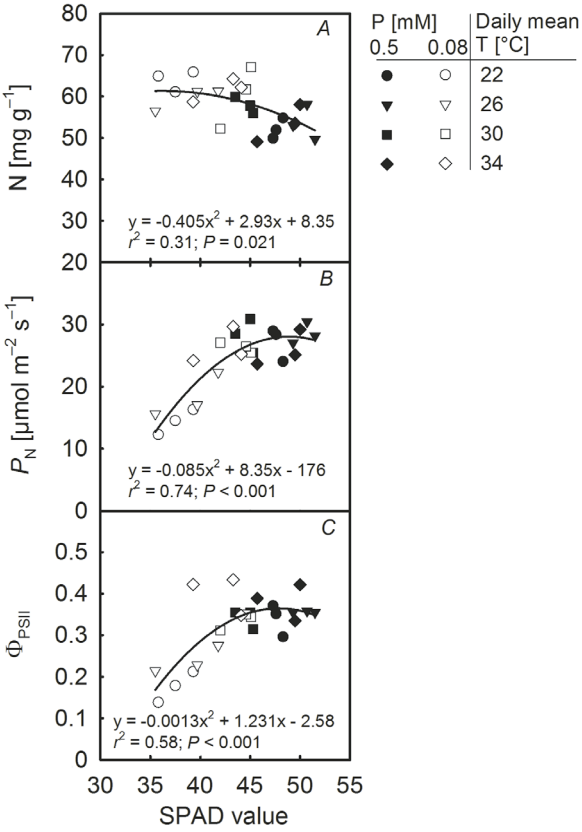

Fig. 5. The regression relationship of SPAD value with (*A*) nitrogen concentration (N), (*B*) photosynthetic rate (P_N), and (*C*) photochemical quantum yield of PSII (Φ_{PSII}) in the uppermost fully expanded leaves of soybean grown under four temperature (T) regimes and two phosphorus (P) nutrition. Symbols represent the individual plant. The line represents the fit of "polynomial second order function" and coefficients of the regression equation is also given. The significance level (P-value) of the regression relationship is also shown.

temperature regimes in P-deficient plants, soybean was able to maintain photosynthetic processes (*e.g.*, P_N, V_{Cmax}, SPAD value, Φ_{PSII}) close to the values observed under sufficient P fertilization, exhibiting a distinctive response. Furthermore, P-deficient plants also exhibited lower stomatal limitation to P_N and markedly higher P and N utilization efficiencies at warmer than OT regimes. Thus, the hypothesis that "plant response to P nutrition will vary when grown under different temperature regimes" was largely validated.

Tissue P and N concentrations: Relative to P-sufficient condition, under the P deficiency, leaf P concentration declined over 50% averaged across temperature, but the N concentration increased over 13%. Similar changes in the foliar P and N concentrations in P-deficient plants have been reported previously, and the increased tissue N might be attributed to the excess absorption *via* roots as it was not limiting in the nutrient solution (Fleisher *et al.* 2012, Singh *et al.* 2014). Under P-limiting conditions, excess N in leaf tissues is often stored as nonfunctional protein and nitrogenous compounds such as amino acids (Israel and Rufty 1988, Staswick *et al.* 1991). Although there were slight increases in the leaf P and N concentrations at the warmer than OT, the effect of temperature was statistically not significant. However, previous studies suggested an increased tissue N or P concentration at warmer temperatures in wheat, especially at the later stages of the development (Manoj *et al.* 2012). The tissue N accumulation under P deficiency resulted into the increased N/P ratio, which is also considered as an indicator of P limitation to the plant growth (Koerselman and Meuleman 1996, Singh *et al.* 2014). The observed foliar P concentration under the sufficient P fertilization was comparable to the observations made in other studies with soybean (Cassman *et al.* 1981, Walker *et al.* 1985).

Photosynthesis processes and nutrient-utilization efficiency: Under P deficiency, the decreased photosynthetic (P_N, V_{Cmax}, g_s, g_m, Φ_{CO2}) and Chl fluorescence (Φ_{PSII}) parameters observed below and at OT was in agreement with other studies (Cure *et al.* 1988, Singh and Reddy 2015). A distinct temperature response of P_N between P treatments was evident due to the P × T interaction. In regards to the T response, under sufficient

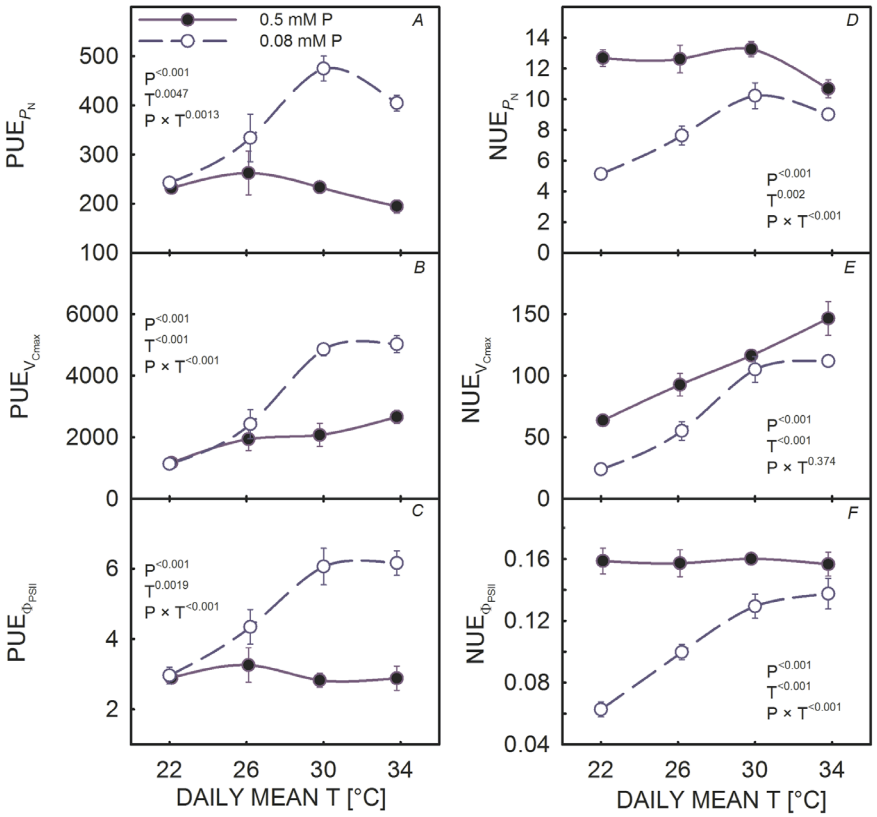

Fig. 6. Response of the phosphorus (PUE) and nitrogen (NUE) utilization efficiencies of (*A*,*D*) photosynthetic rate [PUE$_{PN}$, NUE$_{PN}$; μmol mg^{-1}(P) s^{-1}], (*B*,*E*) maximal rate of carboxylation [PUE$_{VCmax}$, NUE$_{VCmax}$; μmol mg^{-1}(P) s^{-1}], and (*C*,*F*) photochemical quantum yield of PSII (PUE$_{\Phi PSII}$) to temperatures (T) in the uppermost fully expanded leaves of soybean grown under two phosphorus (P) nutrition. Symbols represent the mean ± standard error (n = 3–4). Error bars smaller than the symbol are not visible. The significance level (P-value) of the analysis of variance for the effect of treatments and their interaction (P, T, P × T) is also shown.

P content, P_N tended to decrease at below and above OT, which was consistent with the previous studies in soybean grown under well-fertilized conditions (Sionit *et al.* 1987, Rosenthal *et al.* 2014, Xu *et al.* 2016). In contrast, under P-deficient condition, we found that P_N increased with T and had greater values at warmer T regimes. Remarkably, at warmer than OT in P-deficient plants, P_N was also maintained close to the observation made under sufficient P fertilization. In fact, this maintained P_N at warmer T in P-deficient plants was also supported by the sustained g_s, V_{Cmax}, Φ_{CO2}, Φ_{PSII}, and SPAD values, suggesting a close association among various photosynthetic processes relating to the CO_2 diffusion, carboxylation capacity, photosystem functioning, and Chl. The coregulation of photo-biochemical processes and the CO_2 diffusion inside leaves are vital to optimize carbon fixation and might be considered as an acclimation/adaptation strategy of plants under warmer conditions and nutrient-limited environments (Ort and Baker 2002, Singh and Reddy 2018).

The biochemical limitation (*i.e.*, L_B) is often the primary cause of the photosynthetic limitations in soybean at and below the OT while restricted CO_2 diffusion due to stomatal and mesophyll limitations (*i.e.*, L_S and L_M) play important roles at warmer temperatures under normal conditions (Warren 2008, Xu *et al.* 2016). Under P-deficient condition, a consistent response to temperatures was observed for the photosynthetic limitations, which was primarily attributed to the L_B (49–58%) followed by L_S (31–37%) and L_M (9–20%). In contrast, under sufficient P condition, an exception occurred at the warmest T where L_B and L_S were of the similar magnitude (≈46%) due to a marked increase in L_S while the g_s declined. Rosenthal *et al.* (2014) also reported an increased stomatal limitation at warmer than ambient temperature regardless of the changes in g_s in soybean grown under well-fertilized condition. In the current study, the L_S appeared to limit P_N to a smaller extent under P-deficient condition than under P-sufficient, especially at the warmest T regime. Above OT, an opposite trend between g_m and L_M was found across P concentrations suggesting higher the mesophyll conductance lower the L_M. However, an increased L_M have been reported despite a greater g_m under warmer than optimum temperature regimes in other studies (Warren 2008, Xu *et al.* 2016). The treatment effects on L_B was not statistically significant. Moreover, the insignificant impact of P deficiency on stomatal and mesophyll limitations was in agreement with previous studies in soybean (Singh and Reddy 2016) and tree species (Bown *et al.* 2009, Warren 2011). Thus, under P deficiency, the g_s and g_m at the warmer temperatures either improved or did not restrict the CO_2 diffusion processes, which might have assisted in maintaining the P_N and the carboxylation processes (V_{Cmax}) close to the observation made under P-sufficient condition. The temperature response of V_{Cmax} was consistent between P concentrations and might be considered as an acclimation response of the carboxylation capacity to growing temperatures (Hikosaka *et al.* 2006, Xu *et al.* 2016).

The Chl fluorescence parameters and photosynthetic pigments are used to assess the functional characteristics of the photosystem under stress conditions (van Kooten and Snel 1990, Taiz *et al.* 2014, Singh and Reddy

2015). The similar pattern of Φ_{CO2} and Φ_{PSII} as of the P_N signified a sustained photochemistry of the chloroplasts and adequate functioning of photochemical reactions at warmer temperatures in P-deficient leaves (Singh and Reddy 2018). In fact, the strong association of the Chl content with the photosynthesis and CF parameters (Fig. 5) also delineated the coregulation of these parameters across the treatments to optimize the structural and functional properties of the PSII. A lower SPAD value (as an indicator of Chl content) in P-deficient leaves was observed at and below the OT and might be considered as a plant mechanism to minimize light absorption and photodamage of the PSII (Singh and Reddy 2016, 2018). The Φ_{PSII}/Φ_{CO2} relationship represents quantum yield of PSII activity *vs*. the apparent quantum yield of CO_2 fixation that indicates the PSII activity per CO_2 assimilated (Edwards and Baker 1993, Jacob and Lawlor 1993). The observed increase in Φ_{PSII}/Φ_{CO2} ratio at the warmest temperature might be attributed to the consumption of electrons in processes other than CO_2 fixation including alternative sinks, such as photorespiration, pseudocyclic electron fluxes, and nitrogen metabolism (Edwards and Baker 1993). Evidence of increased photo-respiration under warmer temperatures have been reported previously (Oberhuber and Edwards 1993, Xu *et al.* 2016). Despite the lower CMT in P-deficient leaves at the warmer temperatures, an increased Φ_{PSII} might suggest a thermal acclimation of cellular membranes in leaves adapted to the long-term warmer temperature regime. The observed lower CMT at warmer than OT might indicate an increased fluidity of cellular membranes and was in agreement with the previous reports (Koti *et al.* 2007, Zheng *et al.* 2011). Temperature has a strong influence on the plasma and chloroplast membrane's lipid compositions, and a decreased unsaturation of the cellular membranes under warmer conditions enhances cellular fluidity and thermotolerance where P requiring phospholipids are replaced by the sugar-containing galactolipids (Murakami *et al.* 2000, Zheng *et al.* 2011).

Under P-sufficient condition, intrinsic P-utilization efficiency of P_N (PUE_{PN}) tended to be lower at below or above the OT. A decreased P-use efficiency of grain yield under the warmer (ambient +3 °C) growing condition has also been reported in wheat (Manoj *et al.* 2012). In contrast, under P deficiency, a marked increase in the PUE of photosynthetic process (P_N, V_{Cmax}, and Φ_{PSII}) under warmer than OT signified plant's ability to better utilize tissue available P. This might suggest a greater investment of the available pool of the tissue P in the P-containing cellular metabolites (*e.g*., ATP, NADP) and inorganic phosphate recycling, which play critical role in the energy transfer and photosynthetic processes (Geiger and Servaites 1994, Hidaka and Kitayama 2009). Moreover, the NUE of P_N also increased with temperature, especially, under P-deficient condition, indicating better utilization of the foliar N under warmer conditions. Thus, both the greater PUE and NUE at warmer temperatures might have also assisted to the observed increases in the leaf photosynthetic potential of soybean grown under P deficiency at warmer conditions.

In summary, physiological traits, such as CMT, SPAD value, P_N, g_s, Φ_{PSII}, and nutrient-utilization efficiencies, showed P × T interaction, which was attributable to the distinct effects of P deficiency between the temperature regimes. The P deficiency limited P_N and other photosynthetic traits, particularly at and below the OT. However, this limitation vanished at warmer than OT regimes. Physiological mechanisms to overcome the photosynthetic limitation imposed by P deficiency at warmer T regimes included the sustained photobiochemical traits such as carboxylation capacity (*i.e*., V_{Cmax}) and photosystem functions (*i.e*., SPAD value and Φ_{PSII}) and CO_2 diffusion process by reducing stomatal limitation (L_S) while mesophyll limitation (L_M) remained unaffected. In the same situations, a marked increase in nutrient (P and N) utilization efficiency was also found indicating optimization of metabolic processes through better allocation of the available pool of nutrients. Results highlighted that warmer than OT used in this study compensated a decrease in the rate of leaf photosynthesis due to P deficiency despite the lower foliar P concentration of soybean. Thus, in view of predicted changes in climate and fertility stress, the plant photosynthesis-related processes are likely to benefit from a greater than optimum temperature in moderately P-limited environment when grown under well-watered conditions.

References

Baker J.T., Kim S.H., Gitz D.C. *et al*.: A method for estimating carbon dioxide leakage rates in controlled-environment chambers using nitrous oxide. – Environ. Exp. Bot. **51**: 103-110, 2004.

Bernacchi C.J., Portis A.R., Nakano H. *et al*.: Temperature response of mesophyll conductance. implications for the determination of rubisco enzyme kinetics and for limitations to photosynthesis *in vivo* – Plant Physiol. **130**: 1992-1998, 2002.

Bown H.E., Watt M.S., Mason E.G. *et al*.: The influence of nitrogen and phosphorus supply and genotype on mesophyll conductance limitations to photosynthesis in *Pinus radiata*. – Tree Physiol. **29**: 1143-1151, 2009.

Bunce J.A.: Use of the response of photosynthesis to oxygen to estimate mesophyll conductance to carbon dioxide in water-stressed soybean leaves. – Plant Cell Environ. **32**: 875-881, 2009.

Campbell W.J., Allen J.L.H., Bowes G.: Response of soybean canopy photosynthesis to CO_2 concentration, light, and temperature. – J. Exp. Bot. **41**: 427-433, 1990.

Cassman K.G., Whitney A.S., Fox R.L.: Phosphorus requirements of soybean and cowpea as affected by mode of N nutrition. – Agron. J. **73**: 17-22, 1981.

Cordell D., Drangert J.-O., White S.: The story of phosphorus: Global food security and food for thought. – Global Environ. Chang. **19**: 292-305, 2009.

Cure J.D., Rufty T.W., Israel D.W.: Phosphorus stress effects on growth and seed yield responses of nonnodulated soybean to elevated carbon dioxide. – Agron. J. **80**: 897-902, 1988.

Edwards G.E., Baker N.R.: Can CO_2 assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? – Photosynth. Res. **37**: 89-102, 1993.

Fleisher D.H., Timlin D.J., Yang Y. *et al*.: Uniformity of soil-plant-atmosphere-research chambers. – T. ASABE **52**: 1721-1731, 2009.

Fleisher D.H., Wang Q., Timlin D.J. *et al.*: Response of potato gas exchange and productivity to phosphorus deficiency and carbon dioxide enrichment. – Crop Sci. **52**: 1803-1815, 2012.

Geiger D.R., Servaites J.C.: Diurnal regulation of photosynthetic carbon metabolism in C_3 plants. – Annu. Rev. Plant Phys. **45**: 235-256, 1994.

Grassi G., Magnani F.: Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. – Plant Cell Environ. **28**: 834-849, 2005.

Hartman, G.L., West, E.D., Herman, T.K.: Crops that feed the World 2. Soybean—worldwide production, use, and constraints caused by pathogens and pests. – Food Sec. **3**: 5-17, 2011.

Hewitt E.J.: Sand and Water Culture Methods Used in the Study of Plant Nutrition. Tech. Comm. 22. Pp. 187-190. Commonwealth Bureau of Horticulture and Plantation Crops. Bucks, Commonwealth Agricultural Bureau Farnham Royal, Maidstone, Kent. Bucks 1952.

Hidaka A., Kitayama K.: Divergent patterns of photosynthetic phosphorus-use efficiency versus nitrogen-use efficiency of tree leaves along nutrient-availability gradients. – J. Ecol. **97**: 984-991, 2009.

Hikosaka K., Ishikawa K., Borjigidai A. *et al.*: Temperature acclimation of photosynthesis: mechanisms involved in the changes in temperature dependence of photosynthetic rate. – J. Exp. Bot. **57**: 291-302, 2006.

Israel D.W., Rufty T.W.: Influence of phosphorus nutrition on phosphorus and nitrogen utilization efficiencies and associated physiological responses in soybean. – Crop Sci. **28**: 954-960, 1988.

Jacob J., Lawlor D.W.: Extreme phosphate deficiency decreases the *in vivo* CO_2/O_2 specificity factor of ribulose 1,5-bisphosphate carboxylase-oxygenase in intact leaves of sunflower. – J. Exp. Bot. **44**: 1635-1641, 1993.

Jin J., Wang G., Liu X. *et al.*: Interaction between phosphorus nutrition and drought on grain yield, and assimilation of phosphorus and nitrogen in two soybean cultivars differing in protein concentration in grains. – J. Plant Nutr. **29**: 1433-1449, 2006.

Jones H.G.: Partitioning stomatal and non-stomatal limitations to photosynthesis. – Plant Cell Environ. **8**: 95-104, 1985.

Kim S.-H., Gitz D.C., Sicher R.C. *et al.*: Temperature dependence of growth, development, and photosynthesis in maize under elevated CO_2. – Environ. Exp. Bot. **61**: 224-236, 2007.

Koerselman W., Meuleman A.F.M.: The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. – J. Appl. Ecol. **33**: 1441-1450, 1996.

Koti S., Reddy K.R., Kakani V.G. *et al.*: Effects of carbon dioxide, temperature and ultraviolet-B radiation and their interactions on soybean (*Glycine max* L.) growth and development. – Environ. Exp. Bot. **60**: 1-10, 2007.

Lobell D.B., Asner G.P.: Climate and management contributions to recent trends in U.S. agricultural yields. – Science **299**: 1032, 2003.

Manoj K., Swarup A., Patra A.K. *et al.*: Effect of elevated CO_2 and temperature on phosphorus efficiency of wheat grown in an inceptisol of subtropical India. – Plant Soil Environ. **58**: 230-235, 2012.

Marschner H.: Mineral Nutrition of Higher Plants. 2nd ed. Pp. 265-276. Academic Press, Orland 1995.

Martineau J.R., Williams J.H., Specht J.E.: Temperature tolerance in soybean. II. Evaluation of segregating populations for membrane thermostability. – Crop Sci. **19**: 79-81, 1979.

Maxwell K., Johnson G.N.: Chlorophyll fluorescence: A practical guide. – J. Exp. Bot. **51**: 659-668, 2000.

Mitchell R.A.C., Mitchell V.J., Driscoll S.P. *et al.*: Effects of increased CO_2 concentration and temperature on growth and yield of winter wheat at two levels of nitrogen application. – Plant Cell Environ. **16**: 521-529, 1993.

Mittler R.: Abiotic stress, the field environment and stress combination. – Trends Plant Sci. **11**: 15-19, 2006.

Murakami Y., Tsuyama M., Kobayashi Y. *et al.*: Trienoic fatty acids and plant tolerance of high temperature. – Science **287**: 476-479, 2000.

Oberhuber W., Edwards G.E.: Temperature dependence of the linkage of quantum yield of Photosystem II to CO_2 fixation in C_4 and C_3 plants. – Plant Physiol. **101**: 507-512, 1993.

Ort D.R., Baker N.R.: A photoprotective role for O_2 as an alternative electron sink in photosynthesis? – Curr. Opin. Plant Biol. **5**: 193-198, 2002.

Plank C.O.: Plant Analysis Reference Procedures for the Southern Region of the United States: Southern Cooperative Series Bulletin #368. Pp. 68. University of GA., Athens, GA 1992.

Rogers G.S., Payne L., Milham P., Conroy J.: Nitrogen and phosphorus requirements of cotton and wheat under changing atmospheric CO_2 concentrations. – Plant Soil **155**: 231-234, 1993.

Römheld V., Kirkby E.: Research on potassium in agriculture: needs and prospects. – Plant Soil **335**: 155-180, 2010.

Rosenthal D.M., Ruiz-Vera U.M., Siebers M.H. *et al.*: Biochemical acclimation, stomatal limitation and precipitation patterns underlie decreases in photosynthetic stimulation of soybean (*Glycine max*) at elevated [CO_2] and temperatures under fully open air field conditions. – Plant Sci. **226**: 136-146, 2014.

Ruiz-Vera U.M., Siebers M., Gray S.B. *et al.*: Global warming can negate the expected CO_2 stimulation in photosynthesis and productivity for soybean grown in the midwestern united states. – Plant Physiol. **162**: 410-423, 2013.

Sanglard L.M.V.P., Martins S.C.V., Detmann K.C. *et al.*: Silicon nutrition alleviates the negative impacts of arsenic on the photosynthetic apparatus of rice leaves: an analysis of the key limitations of photosynthesis. – Physiol. Plantarum **152**: 355-366, 2014.

Sharkey T.D., Bernacchi C.J., Farquhar G.D., Singsaas E.L.: Fitting photosynthetic carbon dioxide response curves for C_3 leaves. – Plant Cell Environ. **30**: 1035-1040, 2007.

Sinclair T.R.: Mineral nutrition and plant growth response to climate change. – J. Exp. Bot. **43**: 1141-1146, 1992.

Singh S.K., Reddy V.R., Fleisher H.D., Timlin J.D.: Growth, nutrient dynamics, and efficiency responses to carbon dioxide and phosphorus nutrition in soybean. – J. Plant Int. **9**: 838-849, 2014.

Singh S.K., Reddy V.R.: Response of carbon assimilation and chlorophyll fluorescence to soybean leaf phosphorus across CO_2: Alternative electron sink, nutrient efficiency and critical concentration. – J. Photochem. Photobio. B **151**: 276-284, 2015.

Singh S.K., Reddy V.R.: Methods of mesophyll conductance estimation: its impact on key biochemical parameters and photosynthetic limitations in phosphorus stressed soybean across CO_2. – Physiol. Plantarum **157**: 234-254, 2016.

Singh S.K., Reddy V.R.: Co-regulation of photosynthetic processes under potassium deficiency across CO_2 levels in soybean: mechanisms of limitations and adaptations. – Photosynth. Res. **137**: 183-200, 2018.

Sionit N., Strain B.R., Flint E.P.: Interaction of temperature and CO_2 enrichment on soybean: Photosynthesis and seed yield. – Can. J. Plant Sci. **67**: 629-636, 1987.

Staswick P.E., Huang J.-F., Rhee Y.: Nitrogen and methyl jasmonate induction of soybean vegetative storage protein genes. – Plant Physiol. **96**: 130-136, 1991.

Stocker T.F., Qin D., Plattner G-K. *et al*. (ed.): IPCC. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Pp. 1535. Cambridge University Press, Cambridge and New York 2013.

Suriyagoda L.D.B., Ryan M.H., Renton M., Lambers H.: Adaptive shoot and root responses collectively enhance growth at optimum temperature and limited phosphorus supply of three herbaceous legume species. – Ann. Bot.-London **110**: 959-968, 2012.

Taiz L., Zeiger E., Møller I.M., Murphy A.: Plant Physiology and Development. Pp. 761. Sinauer Associates, Inc., Sunderland 2014.

Taub D.: Effects of rising atmospheric concentrations of carbon dioxide on plants. – Nat. Educ. Knowledge **3**: 21, 2010.

Timlin D.J., Naidu T.C.M., Fleisher D.H., Reddy V.R.: Quantitative effects of phosphorus on maize canopy photosynthesis and biomass. – Crop Sci. **57**: 3156-3169, 2017.

van Kooten O., Snel F.H.J.: The use of chlorophyll fluorescence nomenclature in plant stress physiology. – Photosynth. Res. **25**: 147-150, 1990.

Walker W.M., Raines G.A., Peck T.R.: Effect of soybean cultivar, phosphorus and potassium upon yield and chemical composition. – J. Plant Nutr. **8**: 73-87, 1985.

Warren C.R.: Does growth temperature affect the temperature responses of photosynthesis and internal conductance to CO_2? A test with *Eucalyptus regnans*. – Tree Physiol. **28**: 11-19, 2008.

Warren C.R.: How does P affect photosynthesis and metabolite profiles of *Eucalyptus globulus*? – Tree Physiol. **31**: 727-739, 2011.

Xu G., Singh S.K., Reddy V.R. *et al*.: Soybean grown under elevated CO_2 benefits more under low temperature than high temperature stress: Varying response of photosynthetic limitations, leaf metabolites, growth, and seed yield. – J. Plant Physiol. **205**: 20-32, 2016.

Zheng G., Tian B., Zhang F. *et al*.: Plant adaptation to frequent alterations between high and low temperatures: remodeling of membrane lipids and maintenance of unsaturation levels. – Plant Cell Environ. **34**: 1431-1442, 2011.