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BRIEF COMMUNICATION

Fertilization regimes under hot conditions alter photosynthetic response of bean plants

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Abstract

We examined the effects of foliar application of various nitrogen (urea) concentrations on gas-exchange and chlorophyll (Chl) fluorescence characteristics in bean plants treated by heat stress (42/30ºC, day/night temperatures). Heat stress caused reductions in contents of Chl *a*, Chl *b,* and in maximum photochemical efficiency of PSII by 13, 20, and 27%, respectively, regardless of the N treatment. However, N fertilization caused significant increases in these parameters, especially at higher N concentrations. The net photosynthetic rate and stomatal conductance were enhanced by 32, 60, and 69% and by 25, 88, and 100% due to addition of 5, 10, and 15 mM N, respectively. However, gas-exchange parameters were reduced by 24% due to heat stress. N fertilization alleviated adverse effects of heat stress.

Additional key words: heat stress, nitrogen fertilization, photosynthetic performance, *Phaseolus vulgaris* L.

Various physiological damages occur in plants upon exposure to heat stress (Hasanuzzaman *et al*. 2013). High temperatures (heat stress, HS) are known to inhibit enzyme activity, net photosynthetic rate (P_N), and other metabolic processes leading to physiological and biochemical alterations (Salvucci and Crafts-Brander 2004, Hassan, 2006, Zhao *et al*. 2008, ICPP 2014). HS uncouples enzymes and metabolic pathways leading to accumulation of harmful reactive oxygen species (ROS) which are responsible for oxidative stress (Sarieva *et al*. 2010). The reaction centers of PSI and PSII in chloroplasts are major sites of ROS generation (Chakraborty *et al*. 2011). Stomatal closure, reduction in root growth, and consequent inhibition of mineral uptake are common responses to HS (Huang and Xu 2000).

Nitrogen as an essential element (Sánchez *et al.* 2004, Cechin and De Terezinha 2004) promotes metabolic activities, such as photosynthesis, antioxidant activities, leading to enhancing in growth and yield (Zhao *et al.* 2008).

Due to their different physiological effects, HS and N fertilization might have interactive impacts on plants. HS reduces the rate of nutrient uptake; however, this effect could be mitigated by excessive supply of N (Altenbach *et al*. 2003, Zhao *et al*. 2008, Demmig-Adams *et al*. 2014). Moreover, Zhang *et al*. (2014) reported that N nutrition control is an important way to enhance tolerance to abiotic stress. Thus, it is important to understand how the supply of N may mitigate HS.

The aim of the present experiment was to study the effects of N-fertilization on alleviation of the negative impact of HS on Chl fluorescence, P_N, and stomatal conductance (g_s) of French bean plants.

Seeds of French bean (*Phaseolus vulgaris* L.) were obtained from the Department of Agronomy, Alexandria University, Egypt. They were sown in plastic pots (20 cm^2), five seeds in each pot with multipurpose compost in a heated glasshouse [temperature of 24/20ºC day/night, air humidity 60–65%, 16-h photoperiod with additional irradiance of 450 μmol(photon) m^{-2} s^{-1}, total irradiance was 975 μmol(photon) m^{-2} s^{-1}]. After appearance of expanded trifoliate leaf, 12 days after sowing (DAS), plants were thinned to one seedling per pot. Pots were then transferred to eight closed climate controlled growth chambers. Two chambers were used for N fertilization as foliar spray (three concentrations of urea: 5, 10, 15 mM). Two other

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Abbreviations: Chl – chlorophyll; FM – fresh mass; HS – heat stress; P_N – net photosynthetic rate; g_s – stomatal conductance to water vapour; q_p – photochemical quenching coefficient; NPQ – nonphotochemical quenching; F_v/F_m – maximum photochemical efficiency of PSII; ROS – reactive oxygen species; Φ_{II} – effective quantum yield of PSII photochemistry.

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chambers were used only for HT treatment (40–42ºC), and plants were sprayed with distilled water. Two others were assigned to interaction between N-fertilization and HT, while the last two chambers were assigned as control plants (plants sprayed with distilled water and temperature of 25ºC).

Treatment	Abbreviation
5 mM urea	N5
10 mM urea	N10
15 mM urea	N15
5 mM urea + heat stress	HT
Heat stress	N5HT
Control	CT

Temperatures were maintained in both treatments automatically through a controlled system. Plants were sprayed with either urea or distilled water twice a week. There were 15 pots in each chamber. Soil moisture was checked regularly, at a depth of 0.10 m, using digital psychrometer (*Check-It 0622*, *Check-It Electronics*, USA) to prevent drought stress beyond –0.5 MPa; lost water was replenished by bringing the pots to their original mass according to Deng *et al.* (2000). The watering regime continued until the end of the experiment (Basahi *et al.* 2014).

P_N and g_s were measured with a portable infrared gas analyser (*LICOR-6400, LICOR,* Lincoln, NE, USA). Measurements were taken between 10:00–14:00 h for 55 days after appearance of expanded trifoliate leaf at 5-d intervals at 975 µmol m^{-2} s^{-1} PPFD. Automatic, accurate, and stable temperature control was standard and integral with all leaf cuvettes. Peltier elements were mounted to the head along with a heat sink and fans for precise control of temperature over a wide range. Chl fluorescence was measured with a portable photosynthetic efficiency analyser (*PEA, Hansantech Instrumental,* Hardwick, Norfolk, UK) on the same leaves where the gas exchange was evaluated. Leaves were dark adapted for 30 min in leaf-clips before measurements. The ground (F_0), maximum (F_m), and variable (F_v) fluorescence were measured and used for calculations of variable to maximum fluorescence ratio (F_v/F_m). The effective quantum yield of PSII photochemistry (Φ_{PSII}) was calculated when leaf was completely adapted to light as calculated as $\Delta F/F_m' = (F_m' - F_s)/F_m'$, where F_m' and F_s represent the maximum and steady state fluorescence levels in the light-adapted state, respectively (Bencze *et al.* 2014, Thwe *et al.* 2014). Calculation of quenching due to photochemical (q_p), and nonphotochemical (NPQ) dissipation of absorbed light energy were determined according to the equation: $q_p = (F_m' - F_s)/(F_m' - F_0')$ and $NPQ = (F_m - F_m')/F_m'$ (Yordanov *et al.* 1997, 1999, Calatayud *et al.* 2004, Hassan 2006).

Chl was extracted in cold acetone from all leaves on the main stems of three plants per chamber; six plants per treatment were used. The Chl concentration was determined according to Khan and Khan (1994). Extraction was carried out in a dark room (Ismail *et al.* 2014).

Two-way analysis of variance (*ANOVA*) was applied to log-transformed data. PPFD was used as a covariate for P_N and g_s. Significant differences between means were tested by LSD (*Stattgraphics Statistical Package*, *Plus 5.1*, *Informer Technologies*, Inc., USA).

Heat stress decreased contents of Chl *a*, Chl *b*, and the F_v/F_m ratio by 13, 20, and 27%, respectively, regardless of the N treatment (Table 1). However, N fertilization had no significant effect at N5 and N10 on Chl *a* and *b* values of CT plants, while they increased by 26 and 52%, respectively, at N15. On the other hand, F_v/F_m was elevated by 10, 19, and 36% at N5, N10, and N15, respectively. HS caused an overall reduction in Φ_{PSII} and q_p by 29% and 43%, respectively, while it caused an increase of 56% in NPQ (Table 1).

Nevertheless, N treatments ameliorated negative effects of HS on Chl *a*, Chl *b*, and all Chl fluorescence parameters. Moreover, effects of N fertilization were more pronounced in HS plants than in CT ones.

P_N increased by 32, 60, and 69% and by 25, 88 and 100% due to addition of N5, N10, and N15, respectively (Fig. 1). However, these parameters decreased by 24% each due to HS (Fig. 1).

HS-induced reduction in Chl content indicated adverse effects on light-harvesting complex II as most of Chl *b* is localized there (Reynolds *et al.* 2000, Hassan 2006). Moreover, HS could damage chloroplast membranes resulting in reductions in gas-exchange parameters (Berry and Björkman 1980, Xu and Huang 2000, Zhao *et al.* 2008). Recently, Pelligrini *et al.* (2011) associated the

Table 1. Effects of heat stress (HS), different concentration of N (urea in mM) and their combination on Chl content [mg g^{-1}(FM)], the ratio of variable to maximum chlorophyll fluorescence (F_v/F_m), the quantum efficiency of PSII (Φ_{PSII}), photochemical (q_p), and nonphotochemical Chl fluorescence (NPQ). Means in each row not followed by *the same letter* are significantly different from each other at $p < 0.05$.

Parameter	Treatment							
	Control				+HS			
	N [mM]				N [mM]			
	0	5	10	15	0	5	10	15
Chl a	0.863^{a}	0.892^{b}	0.937^{c}	1.094^{d}	0.603^{a}	0.794^{b}	0.903^{c}	0.941^{d}
Chl *b*	0.503^{a}	0.561^{b}	0.647^{c}	0.783^{d}	0.402^{a}	0.473^{b}	0.543^{c}	0.578^{c}
Chl *a*/*b*	1.716^{d}	1.591^{c}	1.448^{b}	1.397^{a}	1.501^{a}	1.564^{a}	1.663^{b}	1.628^{c}
F_v/F_m	0.703^{a}	0.772^{b}	0.836^{c}	0.852^{d}	0.461^{a}	0.498^{a}	0.687^{b}	0.732^{b}
Φ_{PSII}	0.562^{a}	0.611^{b}	0.695^{c}	0.745^{d}	0.302^{a}	0.341^{ab}	0.358^{b}	0.468^{c}
q_p	0.813^{a}	0.825^{a}	0.854^{b}	0.903^{c}	0.502^{a}	0.525^{a}	0.601^{b}	0.783^{c}
NPQ	0.409^{b}	0.401^{b}	0.376^{a}	0.348^{a}	0.603^{c}	0.589^{c}	0.508^{b}	0.447^{a}

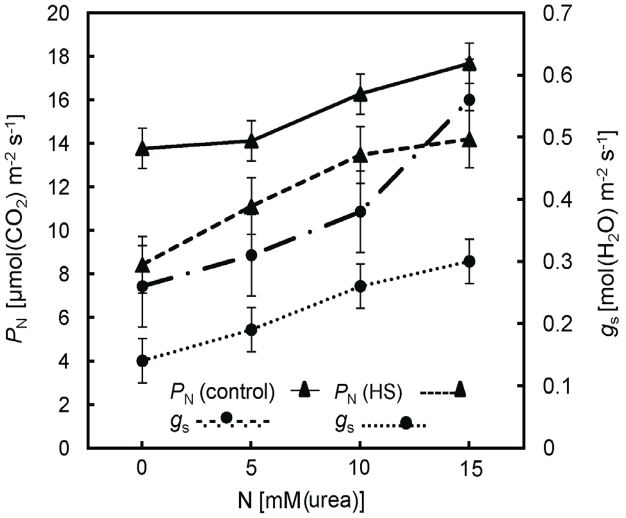

Fig. 1. Effects of different nitrogen (urea) concentrations on net photosynthetic rate (P_N) and stomatal conductance (g_s) under HS (means ± 1 SE).

reduction in Chl contents in stressed plants to inhibition in Chl binding proteins. HS could initiate Chl breakdown directly or indirectly. Such reduction in plant pigment content may represent a possible mechanism to protect the PSII from photoinhibition through a reduction of the number of light-harvesting antennae. However, despite the decrease of the total Chl content, the Chl *a*/*b* ratio remained unchanged. HS induced a decline of the number of functioning photosynthetic units rather than a reduction of the Chl antenna size.

In the present study, N fertilization induced an increase in gas-exchange parameters. This is in agreement with results of Sugiharto *et al.* (1990), who found positive correlation between the photosynthetic capacity of leaves and their N nutrition, suggesting that most of the N is used for the synthesis of components of photosynthetic apparatus (Zhao *et al.* 2008). The reduction in P_N was alleviated by N treatment in HS plants as indicated by significant increases in P_N and g_s.

Maximum photochemical efficiency of PSII (F_v/F_m) is a good indicator of environmental effects on photosynthetic apparatus (Murchie and Lawson 2013). The significant reduction was in agreement with earlier studies (Gesch *et al.* 2003, Hassan 2004). However, the increase in F_v/F_m under N supply could be interpreted as a result of an increase in Chl synthesis (Ciompi *et al.* 1996).

The reduction in q_p, and hence an increase of $(1 - q_P)$, indicated that HS increased excitation pressure on PSII and contributed to the closure of PSII reaction centres, which determined a lower possibility of electron transport from PSII to PSI. In confirmation of that, Φ_{PSII}, closely related to the quantum yield of noncyclic electron transport, decreased in HS-treated plants. Another important finding was the higher value of NPQ in HS-treated plants; they were able to counteract the negative HS effects at chloroplast level because of their capacity to dissipate the excess of excitation energy. HS increases energy dissipation and NPQ, which could lead to a significant reduction in photosynthetic efficiency of PSII (Hassan 2006). Karim *et al.* (2003) reported that mungbean plants grown at low temperature had the highest q_p as well as NPQ compared to the plants developed at high temperature; they concluded that the reduction in NPQ is an indication of loosing photons as heat instead of being used to enhance and promote photosynthesis. This is in agreement with our results. Therefore, N supply helped in maintaining photosynthetic performance relatively high and stable in HS plants, due to increase in Chl content and enhancement in photosynthetic efficiency of PSII in N-supplied beans.

In conclusion, bean plants showed a regulatory adjustment of PSII efficiency, as indicated by its capacity to dissipate the excess of excitation energy (higher NPQ). N fertilization ameliorated negative effects of HS on photosynthesis of bean plants. Moreover, plants became more tolerant to HS. Alleviation of adverse effects of HS by foliar spray of N fertilizer could be used to protect and increase crop production to feed rapidly growing populations in developing countries which have prolonged hot seasons.

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