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Effect of nitrogen regimes on narrowing the magnitude of maize yield penalty caused by high temperature stress in North China Plain

Peng YAN[1], Yuanquan CHEN[1], Adamou DADOUMA[1], Zhiqiang TAO[1,2], Peng SUI[1,*]

[1]*College of Agronomy and Biotechnology, China Agricultural University, Beijing, P.R. China*
[2]*Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, P.R. China*
**Corresponding author: suipeng@cau.edu.cn*

ABSTRACT

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Further enhancement of maize (*Zea mays* L.) productivity will benefit from a thorough understanding of thermotolerance. The effects of nitrogen fertilization regimes (ratio of nitrogen (N) doses prior to planting: V7:V15:R3) on reducing yield penalty imposed by high temperature stress are discussed in this study. Field experiments were conducted in 2013 and 2014 using three nitrogen fertilization regimes (N1 – 120:180:0:0; N2 – 60:90:150:0; N3 – 60:90:60:90) and CK (control) treatment (1:0:0:0) to discuss the effect of nitrogen fertilization regimes on alleviating high temperature stress of spring maize. Total N rates for 2013 and 2014 were 280 and 300 kg/ha, respectively. Yield in 2013 and 2014 was averaged as 9.37 and 12.35 t/ha for N3, respectively, which was 13.47% higher than CK. During the grain-filling stage, leaf area index and the SPAD (soil plant analysis development) value in N3 were the highest, but electrical conductivity and malondialdehyde content of ear leaf in N3 were the lowest. Moreover, photosynthetic rate of ear leaf in N3 increased by 9.95% compared to CK. These results indicate that nitrogen fertilization regimes, especially with N3 treatment, can help maintain relatively higher photosynthetic supply capacity during the grain-filling stage under high temperature stress, thereby resulting in improved grain yield.

Keywords: heat stress; climate change; macronutrient; leaf senescence; photosynthetic capacity

Maize (*Zea mays* L.) is the primary cereal crop in China, accounting for the largest planting area and total yield. Presently, high temperature stress (HTS) at grain-filling stage is one of the main limiting factors for maize production in North China Plain (NCP), especially in the southern part of NCP (Zheng et al. 2001, Li et al. 2003, Tao et al. 2016). The context of climate change implies that crops will be more frequently exposed to a relatively higher temperature in the future (Lobell and Burke 2008, Battisti and Naylor 2009). Therefore, the study of approaching agronomic methods to alleviate HTS at grain-filling stage to achieve high yields has become increasingly important.

Previous research reported that HTS during the reproductive stage sharply decreased grain yield and yield components in maize (Cicchino et al. 2010, Edreira et al. 2014). Under HTS, photosynthetic capacity during grain-filling stage is reduced by accelerated leaf senescence and decreased relative chlorophyll content (SPAD) (Yan et al. 2008, Zhou et al. 2010). Furthermore, the leaf content of malondialdehyde (MDA) accumulated in maize subjected to HTS, caused

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membrane lipid peroxidation and resulted in an increase in the relative electrical conductivity (EC) (Ismail and Hall 1999). Net photosynthetic rate (P_n) decreased by 50–60% in maize under high temperature conditions of 35/30°C (day/night) and 40/35°C (day/night) compared to that under low-temperature conditions of 25/20°C (day/night) and 30/25°C (day/night) (Ben-Asher et al. 2008). In summary, HTS accelerated leaf senescence, increased enhanced maintenance respiration costs, and reduced organic matter accumulation and final grain yield.

Previous studies or practices exploring increased tolerance to HTS lacked a balanced approach to both high yield production and HTS tolerance (Passioura 2010). Mid-season nitrogen application provided recovery of early-season nitrogen stress in harvest grain and resulted in maximum or near-maximum yield (Cazetta et al. 1999). However, the relationship between HTS and nitrogen availability was rarely analysed in maize under field conditions. Field experiments on wheat showed that the degree of damage under HTS increased with higher nitrogen availability, consequently resulting in a reduction in final yield (Morris et al. 2006). However, several investigations demonstrated that sensitivity of crop yield to heat stress was increased with nitrogen fertilization in wheat (Altenbach et al. 2003, Zahedi et al. 2004), in barley (Passarella et al. 2008) and in maize (Ordóñez et al. 2015). Maize will be more often subject to HTS in the context of climate changes. It is urgent to make smart decisions on nitrogen fertilization to reduce the magnitude of the penalty on maize produced under HTS.

This study hypothesized that suitable nitrogen fertilization regimes could reduce the magnitude of yield penalty imposed by HTS during grain-filling stage of spring maize. Four nitrogen fertilization treatments were implemented, including the traditional nitrogen application methods (CK) used by farmer on NCP, as well as three nitrogen fertilization regimes (N1, N2, and N3). The objectives of this study were to evaluate whether nitrogen regimes affect the magnitudes of maize yield penalty caused by HTS, and the physiological mechanism behind this. The results may provide good advice for farmers in improving grain yield and benefits.

MATERIAL AND METHODS

Field experiments were conducted at the Wuqiao Experimental Station (37°41'N, 116°37'E) of China Agricultural University in 2013 and 2014. The soil is considered an alluvial salt aquic soil and classified as silty loam (IUSS Working Group WRB 2006). Soil properties are shown in Table 1. The average annual rainfall of this site is 562 mm with a mean annual temperature of 13.1°C.

The experiment was a randomized block design and plot size was 6.5 m wide and 7.5 m long with three replicates, consisting of 12 rows. The cultivar used in this experiment was JH5 (classified as FAO 800), one of the most commonly used maize (*Zea mays* L.) cultivar in NCP, which exhibits high yield, high quality and multi-resistance. Maize was sown on April 14 in 2013 and April 7 in 2014, respectively. Four nitrogen fertilization treatments were implemented: CK; N1; N2, and N3. Details of nitrogen application for the four treatments are shown in Table 2. One week before planting, 52,8 kg P/ha and 199,2 kg K/ha were applied. The row spacing and plant spacing were 0.55 m and 0.26 m, respectively. Final planting density was approximately 70 000 plants/ha, and final plant population was kept at approximately 68 000 plants/ha. Water was supplied to each plot as needed (the soil water content (0–20 cm) and maintained above 60% of the maximum available water in the soil). Maize was harvested on August 12, 2013 and August 11, 2014. Weeds, diseases, and

Table 1. Soil characteristics of the experimental field

Soil layers (cm)	pH	Electrical conductivity (s/m)	Bulk density (g/cm^3)	Total nitrogen	Organic carbon	Soil mechanical composition (%)		
						sand	silt	clay
				(g/kg)		> 0.05 mm	0.05~0.002 mm	< 0.002 mm
0–10	8.2	0.091	1.41	0.54	4.5	24	60	16
10–20	8.2	0.066	1.47	0.53	4.0	31	56	13

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Table 2. Schedule of nitrogen application at different maize growth stages (percentage) of four nitrogen treatments in 2013 and 2014

Year	Treatment	Basal fertilizer	Jointing stage (V7)	Pre-filling stage (V15)	Post-filling stage (R3)	Total
		(kg/ha)				
2013	CK	300 (100%)	–	–	–	300 (100%)
	N1	120 (40%)	180 (60%)	–	–	300 (100%)
	N2	60 (20%)	90 (30%)	150 (50%)	–	300 (100%)
	N3	60 (20%)	90 (30%)	60 (20%)	90 (30%)	300 (100%)
2014	CK	280 (100%)	–	–	–	280 (100%)
	N1	112 (40%)	168 (60%)	–	–	280 (100%)
	N2	56 (20%)	84 (30%)	140 (50%)	–	280 (100%)
	N3	56 (20%)	84 (30%)	56 (20%)	84 (30%)	280 (100%)

pests were well-controlled throughout the growing season.

Meteorological data were automatically recorded using the HL-10 automatic weather station (Jauntering International Corporation, Taiwan) located approximately 700 m from the experiment field. Daily maximum air temperature, cumulative solar radiation, and cumulative precipitation were measured.

Fifteen plants in the central rows were tagged for measurements at V7 stage. Length (from ligule to leaf tip) and width (the widest portion of the leaf table) of each green leaf were measured *in situ*. The individual green leaf area was estimated using the following formula: leaf area = leaf length × maximum breadth × a constant coefficient (0.5 for not fully expanded leaves and 0.75 for fully expanded leaves). Leaf area index was calculated by summing the individual leaf area divided by plant land area.

Soil plant analysis development (SPAD) was measured using Minolta SPAD 502 chlorophyll meter (Minolta Camera, Co., Ltd., Osaka, Japan). SPAD readings were taken at the central of the upper leaf before silking. During grain-filling stage, the reading was done at the ear leaf. Ten plants per plot were measured at each sampling time.

Electrical conductivity was measured using the following method and the product of EL30 (Mettler Toledo Instruments Inc., Co., Ltd., Greifensee, Switzerland). Ear leaf samples were collected on 0, 10, 20, 30, 40, 50 days after silking in the field at noon. The samples were then brought into the lab and cut into small parts of approximately 1 cm^2 and added to test tube containing 20 mL deionized water. The tubes were then maintained at 25°C for 4 h before electrical conductivity of initial medium (EC1) was determined. These samples were transferred to a water bath at 100°C for 15 min to expel all electrolytes, after which the samples were cooled to room temperature and final electrical conductivity (EC2) was measured. EC was calculated with the following formula:

$$EC = EC1/EC2$$

Malondialdehyde content was assayed with thiobarbituric acid colorimetry (Shalata and Neumann 2001). A 0.5 g ear leaf sample was homogenized in 5 mL 10% trichloracetic acid. The homogenate was centrifuged at 4000 rpm for 15 min, and the supernatant collected; 2 mL of the supernatant and 2 mL of 0.6% thiobarbituric acid were added and mixed in a tube, then heated at 100°C for 15 min and then quickly cooled in the ice box to room temperature. Next, after being centrifuged at 4000 rpm for 15 min, the absorbance of the supernatant was measured at 532 nm and 450 nm.

Measurements of ear leaf net photosynthetic rate were conducted using a Li-6400 photosynthesis system (LI-6400, Li-Cor Inc., Lincoln, USA). Measurements started at 11:00 A.M. on sunny days on five plants in the centre role of each plot. Measurements were conducted at vegetative stage tassel (VT), milk stage (R3) and physiolocal maturity R6 stages, respectively.

At harvest, 6.0 m^2 in the central two rows were extracted in each plot to measure final yield. Ten ears were collected as per size ratio of all ears to determine the final grain number per ear and

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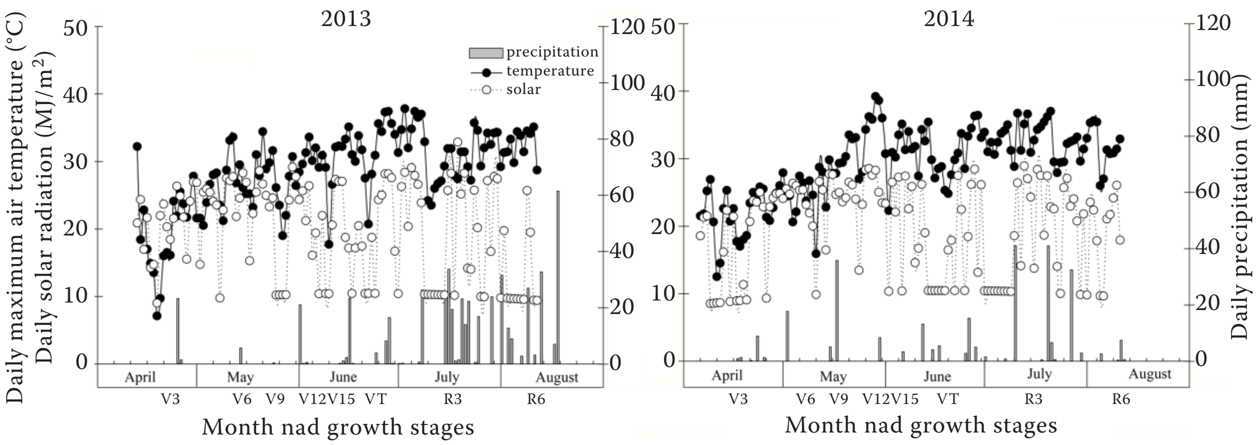

Figure 1. Daily maximum air temperature, daily solar radiation and daily precipitation during the maize growing seasons in 2013 and 2014 at Wuqiao county (37°41'N, 116°37'E), Hebei Province, China

1000-kernel weight. Grain yield was adjusted to 13.5% moisture concentration.

Statistical analyses were performed with SPSS 18.0 software (SPSS, Inc., Chicago, USA). Means were tested using the least significant difference tests at $P < 0.05$ level ($LSD_{0.05}$). Tables and figures were prepared using Microsoft Excel 2010 (Microsoft Corporation, Redmond, USA) and Sigmaplot 12.5 (Systat Sowtware, Inc., San Jose, USA), respectively.

RESULTS AND DISCUSSION

Growing conditions. Average daily maximum air temperature and cumulative solar radiation during the whole growth stages were very similar in 2013 and 2014 (28.5°C, 2411.2 MJ/m and 29.2°C, 2478.9 MJ/m^2 in 2013 and 2014, respectively) (Figure 1). Cumulative precipitation from sowing to maturity was 506.4 mm in 2013 (close to annual average in this region) but only 268.9 mm in 2014.

During grain-filling stage, the average daily maximum air temperature was 31.7°C in 2013, by 0.1°C higher than 2014. Cumulative solar radiation was 1162.5 and 1178.1 MJ/m^2 in 2013 and 2014, respectively. Cumulative precipitation during this stage was 426.4 mm in 2013, it caused a rainy and sunless weather condition; by contrast, cumulative precipitation was only 172.1 mm in 2014 and brought more sunny days, as well.

Yield and yield components. All N fertilization regimes significantly increased grain yield,

Table 3. Yields and yield components under different fertilization regimes

Year	Treatment	Ear number/ha	Kernel number/ear	1000-kernel weight (g)	Yield (kg/ha)	Harvest index
	CK	61688.31^a	430.63^c	318.49^b	8672.04^b	0.40^b
	N1	62987.01^a	451.35^b	327.70^a	9178.56^a	0.43^a
2013	N2	61688.31^a	468.58^a	330.47^a	9252.81^a	0.43^a
	N3	62012.99^a	480.18^a	334.83^a	9365.87^a	0.43^a
	P-value	0.39	< 0.001	0.03	0.001	< 0.001
	CK	60015.74^a	528.00^c	332.65^b	10384.02^c	0.46^b
	N1	60999.61^a	540.87^b	342.96^a	11146.96^b	0.51^{ab}
2014	N2	61491.54^a	546.88^b	348.29^a	11946.88^a	0.51^{ab}
	N3	60507.67^a	555.92^a	351.59^a	12351.71^a	0.54^a
	P-value	0.66	< 0.001	0.02	0.001	0.11

Within each year, means followed by different letter in a column are significantly different at the 0.05 probability level

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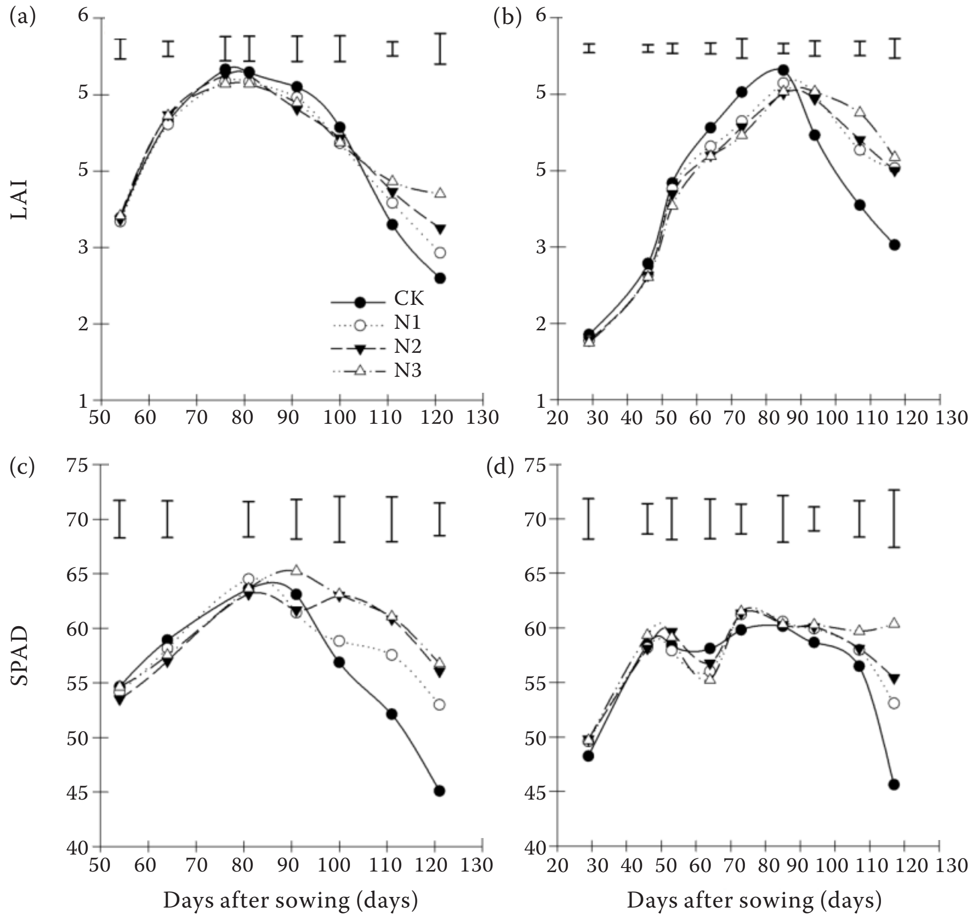

Figure 2. Leaf area index (LAI) and soil plant analysis development (SPAD) during the spring maize growing season under four nitrogen (N) treatments in 2013 and 2014. (a, b) present LAI in 2013 and 2014; (c, d) present SPAD in 2013 and 2014. The vertical bar represents the $LSD_{0.05}$ value

relative to CK (Table 3). However, the increase in grain yield varied with nitrogen application times. Yield of N3, N2 and N1 increased by an average of 13.47, 10.87, and 6.59%, respectively, compared to CK in the two experimental years. N applied at post-silking (N3 and N2) exhibited the maximum effectiveness in reducing the magnitude of yield penalty imposed by HTS, but no significant difference was found between N3 and N2. An increase in grain yield under different fertilization regimes was attributed to an increase in kernel number per ear and 1000-kernel weight. Kernel number per ear under N3, N2 and N1 increased by 8.40, 6.19 and 3.62, and 1000-kernel weight increased by 5.41, 4.23 and 3.00% in N3, N2, and N1, respectively, compared to CK. No significant difference was found in ear numbers among four treatments in both experimental years.

Yield improvement in temperate maize is attributed to greater high temperature stress tolerance, N uptake and associated N efficiency. However, these results were, in contrast to previous reports that observed that sensitivity of yield to heat stress, increased with the level of N availability in wheat (Altenbach et al. 2003, Zahedi et al. 2004), in barley (Passarella et al. 2008) and in maize (Ordóñez et al. 2015). On the one hand, these previous studies were conducted under more or less controlled environments which therefore extended the magnitude of HTS (for example, the maximum air temperature in Ordonez's experiment exceeded 40°C, which rarely appeared in field conditions). On the other hand, most experiments used polyethylene film to control the environment, which could reduce incident photosynthetically active radiation. Conversely, nitrogen promoted crop plant growth at the vegetative growth stage (Sinclair et al. 1989); thus excessed nitrogen application at this stage resulted in a relatively 'high density group' which had a poor light permeability and high level of interspecies competition. In this

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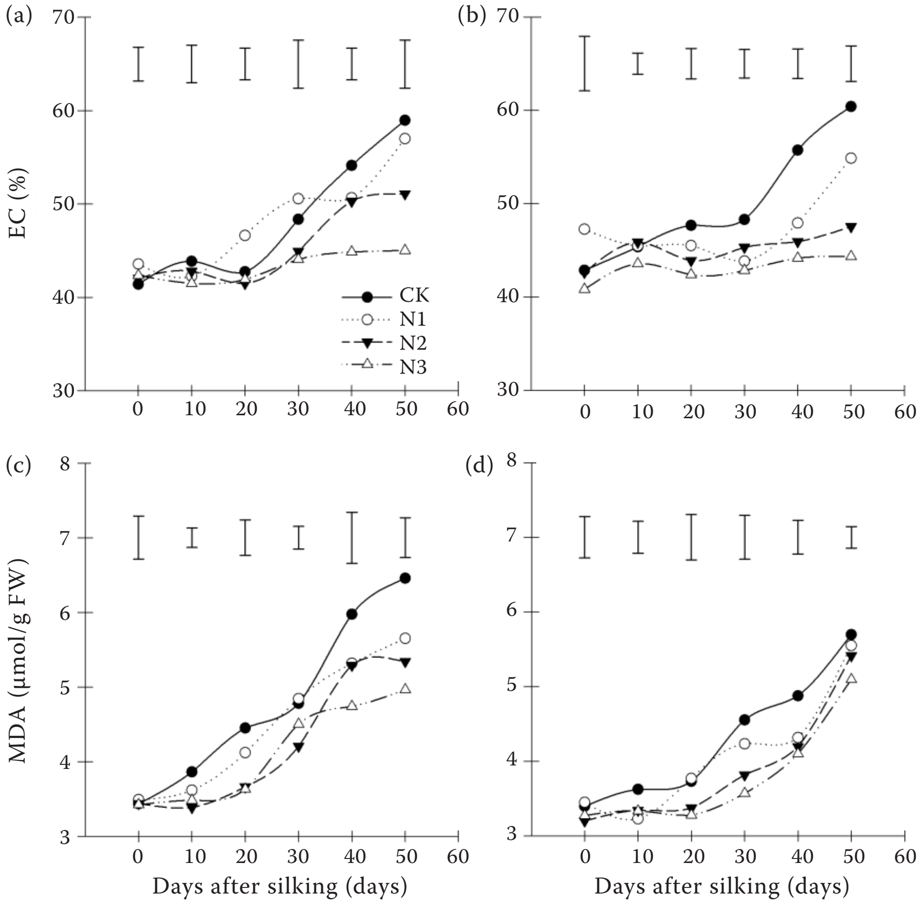

Figure 3. Electrical conductivity (EC) and malondialdehyde (MDA) content of ear leaf after silking (from 0 days to 50 days after silking) under four nitrogen (N) treatments in 2013 and 2014. (a, b) present EC in 2013 and 2014; (c, d) present MDA in 2013 and 2014. The vertical bar represents the $LSD_{0.05}$ value

case, crops are much more sensitive to stressful environments. In the present study, maize was planted in an optimal density according to the previous experiment date, N applied at post-silking stage (N2 and N3) might help relieve HTS at silking and maintain N supply during the grain-filling stage,

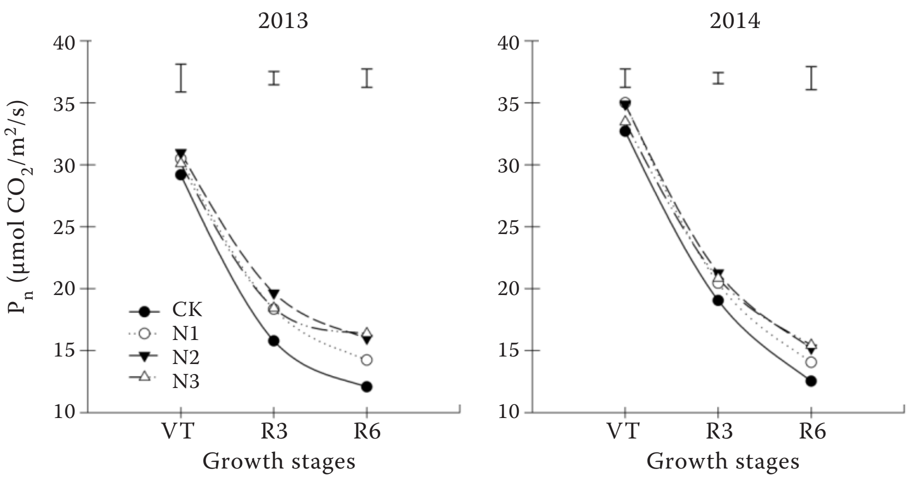

Figure 4. Net photosynthetic rate (P_n) of ear leaf at vegetative stage tassel (VT), milk stage (R3) and physiological maturity (R6) stages under four nitrogen (N) treatments in 2013 and 2014. The vertical bar represents the $LSD_{0.05}$ value

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which resulted in higher kernel number per ear and 1000-kernel weight. These results are consistent with previous studies which showed that mid-season nitrogen application could result in a significant increase in yield (Yan et al. 2014)

LAI and SPAD. Among the four N treatments, CK showed the largest LAI at heading time, increased by 4.63, 3.70, and 3.17% compared to N3, N2, and N1, respectively (Figure 2). However, LAI under CK treatment decreased faster than other treatments from heading to maturity. The N3 treatment showed the largest average LAI during this growth stage, followed by N2 and N1, which increased by 10.70% for N3, 7.59% for N2 and 6.71% for N1 compared to CK, respectively.

No significant difference was found in SPAD at the heading time among the four N treatments. However, similar to LAI, the N3 treatment showed the largest average SPAD during grain-filling stage followed by N2 and N1. The SPAD in CK at harvest was significantly lower than N1–N3; it decreased by 22.56, 18.64 and 14.49% for N3, N2, and N1, respectively. However, this result is contrary to earlier findings which showed that the content of SPAD increased with the growth of nitrogen application to maize (Bullock and Anderson 1998). Since excess nitrogenous fertilizer at vegetative stage led to excessive growth (Jokela and Randall 1989), it reduced the field transmittance rate. Previous studies also showed that increased air and light conditions (reduced plant density) in maize relieved abiotic stress, such as heat stress, shading, drought etc. (Tollenaar and Lee 2002). In the present study, excess nitrogen fertilization before heading increased LAI at heading time significantly and decreased field transmittance rate; in addition, nitrogen could not be held long term in topsoil, and excessive nitrogen application could not delay leaf senescence. As a result, plants in the CK treatment showed a sharp decrease in LAI and SPAD at approximately 30 days after silking.

EC and MDA. No significant differences in EC of ear leaves were observed at silking among the four N treatments in both years (Figure 3). However, EC of ear leaves in CK increased faster than in the other three N treatments 20 days after silking. Post-silking EC of ear leaves in N3, N2, and N1 averaged 43.16, 45.33 and 47.96, respectively, which was approximately 7.46% lower than CK.

Similar to EC, there was no significant difference in MDA of ear leaves among the four N treatments at silking. However, MDA of ear leaves in CK showed a fast growth ten days after silking; by contrast, N3 showed a much slower increasing trend. MDA of ear leaves averaged 3.95, 4.06 and 4.30 for N3, N2, and N1, respectively, which was approximately 10.25% lower than CK. These findings are partly consistent with the results of earlier works which reported that nitrogen supply facilitates the establishment of kernel sink capacity and promotes the activity of enzymes relating to sucrose and nitrogen uptake (Cazetta et al. 1999). Increasing nitrogen supply, especially during the late grain-filling stage prevents nitrogen remobilization from leaves and stems, eventually delaying leaf senescence, helping keep the plant 'stay green'.

Net photosynthetic rate. Net photosynthetic rate (P_n) decreased quickly during grain-filling stage among the four N treatments. At silking, no significant difference in P_n was measured among treatments (Figure 4). However, P_n in CK showed a more rapid decline after silking than did the other N treatments. At harvest, P_n of CK declined to 12.30, which was significantly lower than those of N3, N2, and N1. P_n averaged 21.95, 22.89 and 23.50 for N3, N2, and N1, respectively, which were approximately 13.71% higher than CK. These results are in agreement with Shangguan et al. (2000), who reported that nitrogen deprivation in winter wheat strongly reduced P_n.

The present study provides an approach for assessing the effect of nitrogen fertilization regimes on relieving HTS of maize in field conditions. The use of nitrogen fertilization regimes could significantly reduce the magnitudes of maize yield penalty caused by HTS. With nitrogen fertilization treatment, more nitrogen was applied at growing season rather than prior to planting to ensure maize demand for nitrogen especially during later growth stage, which resulted in delayed leaf senescence (larger average LAI and higher average SPAD) and relatively higher photosynthetic capacity (lower EC and MDA content in ear leaf, higher average photosynthetic rate). The mechanism of the relationship between crop N availability and HTS in field conditions needs to be further studied.

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