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Examination of photosynthetic nitrogen use efficiency of field-grown sweet corn (*Zea mays* L var merit) under water and nitrogen stress

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Abstract

The physiological response to drought stress in sweet corn (*Zea mays* L var merit) is not well understood. Water and nitrogen utilization properties of leaves can play key roles in plant drought tolerance. During the grain-filling period, increasing photosynthesis per unit plant N, namely photosynthetic N-use efficiency (PNUE) is a potential way to increase N-utilization efficiency (NUtE). The objective of this study was to determine whether the PNUE within the canopy is optimized as a method of maximizing canopy productivity under low N conditions and/or water stress. Sweet corn plants were grown in the field in two consecutive years with three N levels (0, 175, and 225 kg N ha^{-1}) and two water regimes (100 and 60 percent field capacity). Throughout the grain-filling period significant interactions of irrigation regime x nitrogen fertilization on specific leaf N (SLN), net photosynthetic rate (P_N), PNUE (calculated as P_N/SLN), stomatal conductance (gs), transpiration rate (E) and green leaf area (GLA) were measured every 10 d after silking (DAS). Significant differences in SLN, P_N, gs, E, and PNUE occurred in leaves. Ear FM without N (N0) (13.69 and 15.56 Mg ha^{-1} across 2 yr) was 37% and 45% and 34% and 41% less than the average of N125 and N225 across both years, but physiological NUtE increased by 21% without N. Correspondingly, PNUE at high levels of N was similar to that of the full irrigation, with both of them having smaller PNUE than the lower of N. In conclusion, improved whole-plant NUtE under water stress and with N0 may both contribute to the high degree of physiological acclimation of sweet corn to drought and the overall increase in PNUE in the leaves.

Keywords: *Zea mays*, sweet corn, drought, nitrogen use efficiency, photosynthesis

Introduction

Improvement of yield stability under conditions of water shortage (Guo, 2013) will require a better understanding of delayed leaf senescence or «stay green» (SG) during the grain filling phase, awareness of the functional significance of the photosynthetic and nitrogen remobilization phases of leaf development (Guo, 2013) and a better understanding of leaf senescence during stress (Guo and Gan, 2012). Nitrogen fertilizers have been used extensively to increase crop yields. However, crops are only capable of using 30 to 40% of this applied N to enhance grain yield (Chen et al, 2016). The interaction between water and nitrogen (N) has been studied for a long time. Drought stress may reduce the availability of N fertilizer and hasten leaf senescence (Herrera-Rodríguez et al, 2007). For crops such as sweet corn and sorghum the 'stay green' trait confers better drought tolerance, and some of these varieties were found to be more efficient in N assimilation (Kamara et al, 2014). Nitrogen-utilization efficiency is closely related to N-use efficiency, especially under low N stress (Chen et al, 2016). Photosynthesis is the basis of plant productivity and, hence, crop yield. In maize, almost all the grain dry matter at maturity comes from photosyn-

thates produced during the postsilking stage (Chen et al, 2015). Therefore, NUtE at the whole-plant level during grain filling is reflected by greater photosynthate production per unit plant N, that is, PNUE. Studies on sweet corn in semi-arid regions, where drought frequently occurs, may identify the physiological bases of high productivity under drought conditions (IPCC, 2007). Sweet corn belongs to the NADP-malic enzyme (NADP-ME) subtype of C4 plants that often have a lower leaf nitrogen content and a higher NUE and instantaneous PNUE than do plants of the NAD$^+$-malic enzyme (NADME) subtype (Ghannoum et al, 2005). The high productivity, drought tolerance and nitrogen-use efficiency of sweet corn in the field is widely known, but the physiological relationships underlying these features are not well understood. Physiologically, the relationship between leaf N and PN is well documented. Leaf N content affects P_N via regulation of the electron transfer system and photosynthetic enzymes. Several researchers report that light-saturated CO_2 assimilation rate strongly correlates with N content per unit leaf area (SLN; Hilbert et al, 1991). The dynamics of specific leaf N (SLN, g N m^{-2} leaf) occupies a central position in the issue of functional «stay green» since leaves are an important

source of N for grain filling while. On the other hand, maintenance of leaf photosynthesis in SG cultivars could support the continued absorption of N by the roots. High SLN at silking has been linked to delayed leaf senescence in maize (Kamara et al, 2014). The relationship between maximum photosynthetic capacity and SLN for corn leaves is well established (van Oosterom et al, 2010). By contrast, the relationship between crop NUE and SLN follows theoretical models, especially at the low levels of SLN (Massignam et al, 2009). Approximately 50 to 80% of N in leaves is located in photosynthetic proteins (Kamara et al, 2014). In maize, N deficiency reduces PN by decreasing phosphoenolpyruvate carboxylase activity, chlorophyll content, and soluble protein concentration in leaves (Ding et al, 2005). PN will not further increase when SLN reaches 1.2 to 1.8 g m^{-2}, depending on the environment and genotypes (Chapman and Barreto, 1997). Uribelarrea et al (2009) reported that excess N accumulation in leaves of field-grown maize could not increase leaf P_N because the excess N is channeled into phosphoenolpyruvate carboxylase rather than Rubisco in the leaves. Interactions between water and N stresses under field conditions are obviously quite common in the production of corn (*Zea mays* L). Data has shown that crop response to irrigation depends on the sufficiency of N in the soil. An increase in whole-plant photosynthesis with efficient N and water use may be the key to improving GY and N-use efficiency when other factors are held constant (Long et al, 2006). Several scientists have suggested that there is an optimum N distribution for maximizing canopy photosynthesis (Hikosaka, 2014). Nitrogen partitioning within the plant can be affected by N supplies, leaf age, ear development, specific leaf area and leaf width (Drouet and Bonhomme, 2004). In maize, SLN in leaves was closely related to «stay green» of leaves that is, with high amount of irrigation and also had high N concentration (Drouet and Bonhomme, 2004). Corn cultured in an N deficient soil produced a lower leaf area and maintained a much lower rate of transpiration per plant than plants grown in high soil N (Zuo et al, 2009). During the grain-filling phase, leaf N is remobilized to the grain, with more leaf N mobilized to grain under low N conditions (Chen et al, 2015). Drouet and Bonhomme (2004)

measured P_N and SLN at maize leaf under nitrogen deficiency. The variation of SLN in their experiment was very limited (1-1.6 g m^2) . We analyzed the leaf N, PN, leaf area index and PNUE within the canopy throughout the grain-filling period and evaluated the adaptation to low N and water stress.

The aim of this study was to understand: whether the PNUE within the canopy was optimized together with the SLN, and whether the whole-plant NUtE was improved by the optimization of photosynthesis-related traits within the canopy. To this end, net photosynthesis, transpiration rate and stomatal conductance were measured over two-year on different conditions of nitrogen fertilization and irrigation regime in semi-arid environment.

Materials and Methods

Field experiment

A field experiment was conducted in 2014 and 2015 at Shiraz University (52°02'E;29°56'N, 1850 masl), 12 km north of Shiraz, IRAN. The soil at a depth of 60 cm was sampled before the experiment and subjected to a physicochemical analysis. It was silty clay loam (average of two years). Some physical and chemical characteristics of the experimental soils are provided in Table 1. A soil test showed a pH of 7.50 (1:2 (v/v) soil:water; Blackmore et al, 1987). Experiments 1 and 2 were split plot randomized complete blocks designed with three water regimes [(100 (l1), 80 (l2), and 60 (l3) percent field capacity (FC)] as the main plots and five rates of nitrogen (N) as urea [0 (N1), 75 (N2), 125(N3), 175 (N4), and 225 (N5) kg ha^{-1}] fully randomized in the sub-plots. A single cross sweet corn (*Zea mays* L var merit) seeds were planted. Each sub-plot measured 4 (4 rows) × 3 m with the between row and within row plant spacing of 0.70 and 0.15 m, respectively. Five rates of N applied at different vegetative development (Ritchie et al, 1993). Both experiments had three replicates. Pre-trial soil tests showed that available P and K where moderately high (Olsen P 16 mg kg^{-1}; available K 329 mg kg^{-1}). Even so, base fertilizer of 200 kg triple superphosphate (20.5% P) and 100 kg ha^{-1} potassium chloride (KCl; 52% K) was applied on 18st Jun 2014 to ensure that P or K did not constrain plant growth. Nitrogen treatments were applied in the form of urea (46% N) in

Table 1 - Soil test results from 9nd May 2014 (Experiment 1) and 12th May 2015 (Experiment 2) for the experimental sites at Shiraz University, Shiraz, IRAN.

BD (Mg m^{-3})	FC (cm^3 cm^{-3})	PWP (cm^3 cm^{-3})	OM (%)	Sand (%)	Silt (%)	Clay (%)
1,528	0.31	0.19	0.6	19	48	33

N^a (kg ha^{-1})		N^b (kg ha^{-1})		K (mg l^{-1})	P (mg l^{-1})	EC (dS m^{-1})
2014	2015	2014	2015			
227	245	35.5	46.8	329	16	0.6

BD - bulk density; FC - soil water content at field capacity (-0.03 Mpa); PWP - soil water content at permanent wilting point (-1.5 Mpa); OM - organic matter; N^a - Available N (0.6 m depth); N^b - Mineral N (1.0 m depth); K - potassium in soil extract; P - phosphorus in soil extract; EC - soil electrical conductivity.

split applications in three applications on 2nd July and 22nd Jun (starter), 23th and 12th July (4 Leaves stage) and 14th and 2nd August (7 leaves stage) in 2014 and 2015, respectively. The first N fertilizer was band applied 5 cm under the seeds in the row. The second N fertilizer was side dressed at a 10-cm depth. Crops was seeded on 18 and 10 Jun 2014 and 2015 and harvested on 30 and 19 Sept 2014 and 2015. At the V3 stage, seedlings were thinned to a density of 8 plants m^{-2}, which is the optimum population density for commercial sweet corn in this region. Plots were kept free of weeds, insects, and diseases based on standard practices. To monitor soil moisture extraction, neutron probe access tubes and TDR rods were installed in replicates 2 and 3 to a depth of 1.0 m and 0.2 m, respectively. The trigger for irrigation was set conservatively at 60% of total available soil water (Ritchie et al, 1993). Daily weather was accessed (Figure 1). Total soil residual N plus applied N (SRN) were (35, 110, 160, 210, and 260 kg ha^{-1}) and (46, 121, 171, 221, and 271 kg ha^{-1}) for different N treatments (N1, N2, N3, N4, and N5) in 2014 and 2015, respectively. Full irrigation (I1) was applied to increase the soil water content of root zone to field capacity at 7 days intervals that was estimated according to:

$$Id = \sum_{i=1}^{k} (\theta_{fci} - \theta_i)\Delta z_i$$

where Id is the required amount of full irrigation (m), θ_{fci} and θ_i are the field capacity and water content of soil layer i, respectively (m^3 m^{-3}), k is the number of soil layers and Δz_i is the thickness of each soil layer (m). The amounts of irrigation water in I2 and I3 were calculated by multiplying the measured amount of water for I1 by 0.8 and 0.6, respectively. Daily thermal units were accumulated after emergence from air temperatures using the modified sine curve method with a base temperature of 0°C and an optimum temperature of 34°C (Jones and Kiniry, 1986). In this experiment, we just consider the treatments of nitrogen (N0, N125, and N225 Kg N ha^{-1}) and levels of irrigation (I1 and I3) in the characteristics described.

Measurements
It takes 60 to 65 d from seeding to silking and approximately 21 d to reach physiological maturity. Two plants with the same silking date were tagged in each plot. Green leaf area was monitored destructively by harvests of three plants cut at ground level every 15 days interval using a leaf area meter, (AF-Delta-T) starting 30 DAE. (Gallais et al, 2006). Every 10 d from silking until ripening the «stay green» leaves were examined. For a partially stay green leaf, GLA was estimated by multiplying the proportion of the leaf remaining green by the leaf area of that same leaf at silking. The net photosynthetic rate (P_N) was also measured on two plants per plot (eight plants in total for each treatment) between 11:30 to 12:30 every 10 d from silking until ripening. P_N measurements were taken from the two plants on upper leaf (third
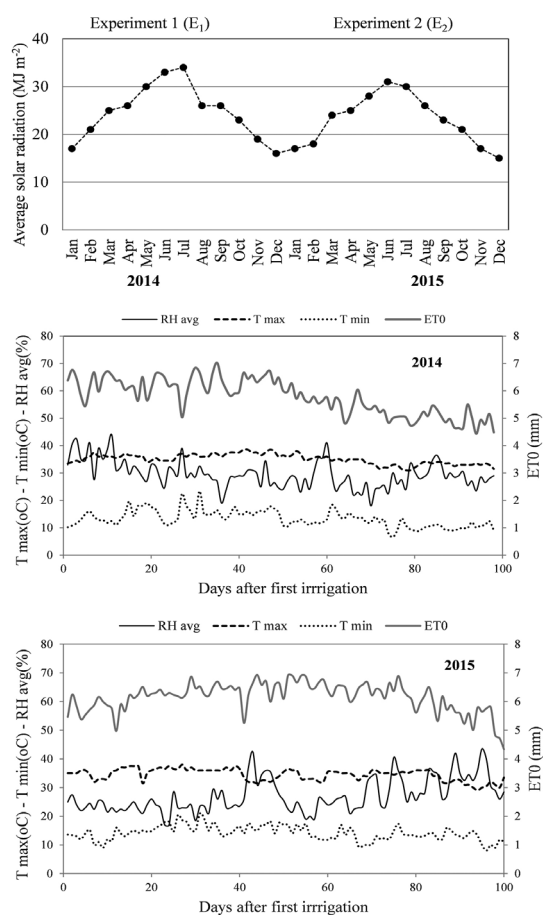

Figure 1 - Weather conditions experienced by sweet corn crops in the first (2014, Experiment 1, E1) and second (2015, Experiment 2, E2) growth seasons.

leaf above ear leaf), avoiding the midrib. Net photosynthetic rate was measured with a portable photosynthesis system (Li6400; LI-COR, Lincoln, NE, USA) coupled with a standard red/blue LED broadleaf cuvette (6400-02B, LI-COR) and a CO_2 mixer (6400-01, LI-COR), under a consistent controlled-light intensity of 1600 µmol m^{-2} s^{-1}. The measurements were obtained at a leaf temperature of 30 ± 0.5°C and a CO2 concentration of 400 ± 1 µmol CO_2 mol air^{-1} inside the chamber (Uribelarrea et al, 2009). It took about 60 to 300 s for maximum P_N of the leaves to become stable after exposure to 1600 µmol photosynthetic-photon-flux-density m^{-2} s^{-1}. At least two measurements were taken on each leaf and more were taken if the first two values varied too much. In addition to P_N data, data for stomatal conductance (gs) were obtained using the portable photosynthesis system at the same time. After P_N and gs measurements, measured leaves (third leaf above ear leaf) were harvested, the main leaf midribs were removed, and the remaining tissue combined. The tissue was then dried at 105°C for 30 min, dried at 70°C to a constant weight, weighed to obtain the dry weight, and then ground into a fine powder. A portion (80 mg) of leaf tissue was used to

Table 2 - Effects of fertilizer N rates and irrigation regime on green leaf area index, N nutrition index, shoot N accumulation, Nitrogen utilization efficiency, and Ear fresh mass at harvest of maize in 2014 and 2015.

treatments[†]		GLAI at silking		Nitrogen nutrition index at silking		Shoot N accumulation at silking (kg ha⁻¹)		Nitrogen utilization efficiency (kg kg⁻¹)		Ear fresh mass (Mg ha⁻¹)	
Year		2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
	N0	3.53d[‡]	3.69d	0.60b	0.66b	37.81c	44.28c	61.2a	68.2a	13.69c	15.56c
I1	N125	5.59ab	5.46ab	0.83a	0.85a	93.53b	106.19ab	53.1b	57.6b	21.89ab	23.92ab
	N225	6.25a	6.30a	0.87a	0.89a	152.65a	159.42a	51.9b	54.2b	24.79a	26.46a
	N0	2.60e	2.88de	0.57b	0.59b	22.48d	25.55c	58.4	60.5	10.82c	10.09c
I2	N125	4.14c	4.14c	0.75a	0.77a	61.85c	72.48b	51.5	53.6	15.13b	17.86b
	N225	4.79b	5.05ab	0.78a	0.82a	87.91b	99.51ab	50.9	51.9	17.42ab	18.35ab

[†]N0, N125, and N225 indicate N application of 0, 125, and 225 kg N ha⁻¹, respectively. I1 and I3 indicate irrigation regime (I) application of 100 (I1) and 60 (I3) percent field capacity (FC). Nitrogen nutrition index was calculated according to Plénet and Lemaire (2000). [‡]Within nitrogen and water treatment, means in the same column followed by different letters indicate significant differences (P < 0.05).

determine total N concentration using a Vario MAX CN element analyzer (Elementar Analysensysteme GmbH). Based on the area, dry weight, and N content of the leaves used for PN measurements, specific leaf area (cm^2 g^{-1}) was calculated as leaf area (cm^2) per dry weight (g^{-1}), specific leaf N (SLN; g N m^{-2}) was calculated as N content (g) divided by whole-leaf area (m^{-2}). Photosynthetic N-use efficiency (µmol CO_2 g^{-1} N s^{-1}) was calculated as P_N/SLN. The P_N by SLN curve for leaves was fitted by applying a logistic equation (Sinclair and Horie, 1989). Instantaneous photosynthetically active radiation (iPAR; µmol m^{-2} s^{-1}) distribution in the canopy was measured at 40 day after sown (DAS) in 2014 and 2015, using a PAR Line Quantum Sensor (Skye Instruments Ltd). The measurements were conducted between 09:00 and 12:00 in three representative rows in each of the experimental plots. Each measurement was repeated three times. The probe was positioned horizontally between two rows, and the iPAR was measured at the canopy top and at ground level. The fraction of intercepted PAR (iPAR) was derived following the method of Gallagher and Biscoe (1978):

PAR_i = 1.0 - PAR_t, where, PAR_t is the fraction of PAR transmitted.

Daily solar radiation (MJ m^{-2} d^{-1}) was obtained from measurements of daily global short wave radiation recorded at Bradfield's' meteorological station, 3 km east of the experimental site. Incident PAR was assumed to equal 0.5 of total incident short wave radiation. Total intercepted PAR was estimated following the procedure of O'Connell et al (2004). Briefly, daily estimates of iPAR between emergence and physiological maturity were made by linear interpolation of the instantaneous measures of PARi with respect to time. Then, daily intercepted PAR (Sa) was calculated:

S_a = PAR_i x S_i, where, S_i is the daily incident PAR.

Daily intercepted PAR was summed from emergence to physiological maturity to obtain the total intercepted PAR. At silking, physiological maturity and maturity, five plants from each plot were cut at the soil

surface and separated into leaves, stalk (leaf sheaths, tassels, husks, and cobs), and total ear fresh mass (FM). Results are presented on a fresh mass basis as this is most relevant for this crop. Harvest index was calculated as the ratio of ear yield over fresh weight per plant to total plant fresh biomass. All samples were dried at 105°C for 30 min, dried at 70°C to a constant weight, weighed to obtain the dry weight, and then ground into a fine powder. A portion (80 mg) of leaf tissue was used to determine total N concentration using a Vario MAX CN elemental analyzer (Elementaranalysen-systeme GmbH).

The N-utilization efficiency (NUtE) was calculated as ear FM per unit of N uptake at maturity (Moll et al, 1982). The N nutrition index was calculated as follows: N nutrition index = N concentration of the plant/ the critical N concentration for sweet corn. The critical N concentration (g kg⁻¹) was calculated by 34.0 × the dry matter (Mg ha⁻¹) -0.37 as suggested by Plénet and Lemaire (2000).

Statistical analyses were performed using the software R. Analysis of variance (ANOVA) was used to partition the observed variation between treatment effects and errors. For analysis with significant results (P-value of the ANOVA was less than 0.05) a Duncan's LSD (least significant difference) *post hoc* test was performed out.

Results

Yield formation and nitrogen utilization efficiency

Ear FM, shoot N accumulation, and green leaf area index responses to N varied with irrigation regime (I1 - I3) and under N225 were all greater in 2014 than in 2015; the differences were larger among N treatments in 2015 than in 2014 (data not shown). This difference in NUtE was also affected by irrigation × N interaction. The N nutrition index with N0 was only 0.61 and 0.68 at silking, respectively in 2014 and 2015 (Table 2). The N0 treatment also reduced N uptake and canopy size; the latter as reflected by the maximum green leaf area index. Ear fresh mass
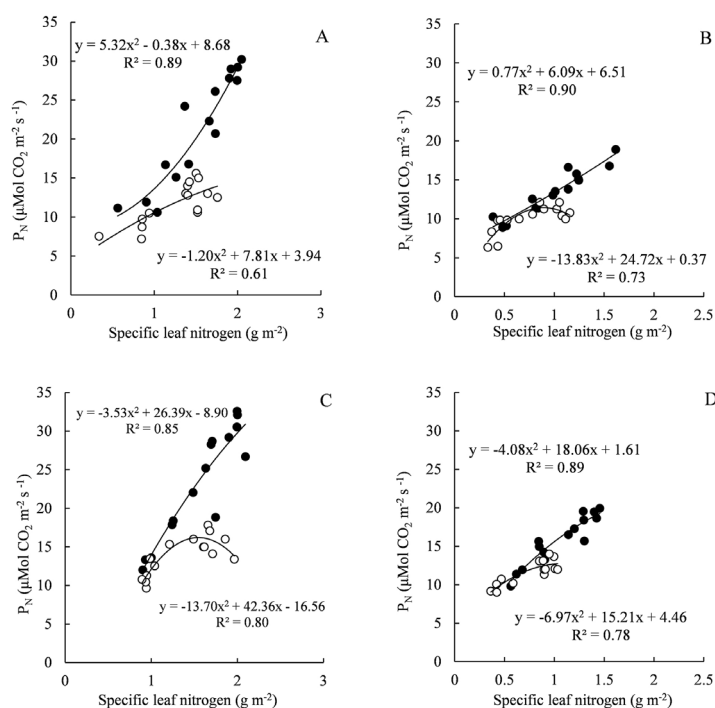
Figure 2 - Correlation between net photosynthetic rate (PN) and specific leaf N content (SLN) at two irrigation regime (I100 (•) and I60 (○) % FC) and five N treatments (N0, N75, N125, N175 and N225 kg ha-1N) in 2014 (A, B) and 2015(C, D) at silking and physiological maturity.

(FM) differed among N treatments (Table 2). Ear FM with N125 (21.89 and 23.92 Mg ha⁻¹) was similar to that with N225 (24.79 and 26.46 Mg ha⁻¹) in 2014 and 2015, respectively. These similar between ear yields are typical of those in the experimental region. Ear FM without N (N0) (13.69 and 15.56 Mg ha⁻¹ across 2 yr) was (37% and 45 %) and (34% and 41%) less than the average of N125 and N225 across 2 yr. The negative impact of water stress on Ear FM found out in this experiment was in agreement with Bruce et al, 2002. The relative decreases in green leaf area index N0 were much smaller than the decreases in Ear FM and N accumulation, indicating a more efficient use of limited N for leaf growth and grain formation with the low N rate. Accordingly, NUtE with N0 was 21% greater than the average of N125 and N225 across 2 yr. Until the silking, the increase in GLAI was constant and linear according to the irrigation management with the maximum value of 6.25, 5.59 and 6.30, 5.46 m2m-2 as the means in full irrigation treatment (I1) for 2014-2015, respectively Table 2. The water stress (I3) reduced the maximum LAI of N225 and N125 0.46%, 0.37% and 0.43%, 0.30% compared to N0 treatment and decline of LAI in I3 was faster than I3. Irrespective for the irrigation water supply, the fast decline of LAI at silking was a constant for all the nitrogen treatments in two years confirming the findings of other studies (Andrade, 1995). Moreover, with starting the decline of LAI, also Shoot N accumulation and translocation of assimilates from leaves to the other

plant organs resulted lowered (Plenet et al, 2000). Green leaf area increased from the lowest amount of irrigation (I3) to the full irrigation and then consistent across N levels (Figure 3). There was no difference in the LAI between the N125 and N225 treatments. The effect of N0 on GLA was weaker than N125 and N225 treatments at I1 and I2 across 2 yr (Table 2).

Specific Leaf Nitrogen, Net Photosynthetic Rate, Photosynthetic Nitrogen-Use Efficiency, Stomatal Conductance and Transpiration rate

Specific leaf N was significantly affected by N rates and irrigation levels throughout the grain-filling phase in 2 yr (Table 3). The SLN values under N125 and N225 at full irrigation (I1) were similar, with both greater than that under N0 in 2 yr. Water stress can significantly reduce SLN values and were affected by N level. The SLN of water stressed sweet corn increased more slowly and was significantly lower than full irrigation in the period of grain filling on N125 and N225 compared with N0. Net photosynthesis rates for the different irrigation treatment were consistent across N rates (Table 4). The PN trend at two irrigation levels and three N rates was similar to that of SLN. This was associated with faster leaf senescence and decrease GLAI under N0 treatment, which resulted in a complete cease in water stress (I3; Figure 2). The correlations between PN and SLN were evaluated in both years. Generally, there was an exponential correlation between PN and SLN throughout the grain-filling phase (Figure 2). At the same SLN, PN
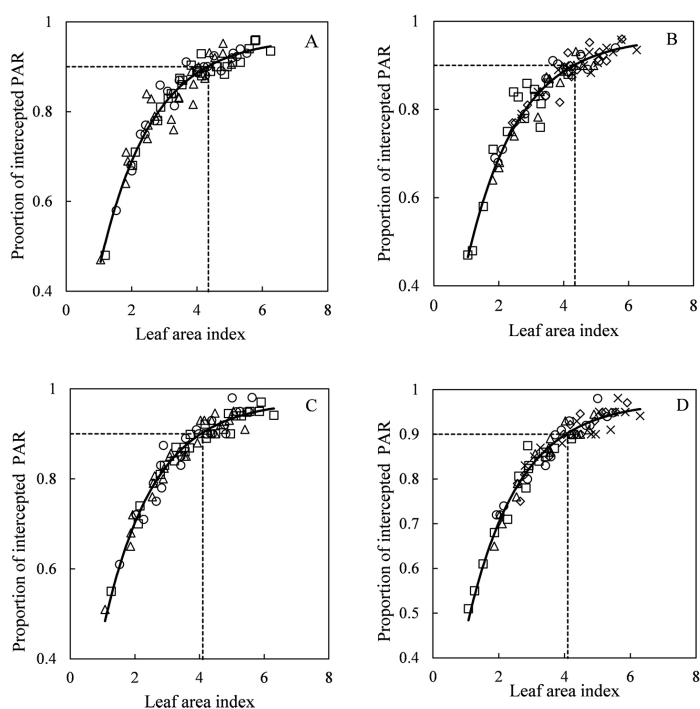
Figure 3 - Proportion of intercepted photosynthetically active radiation versus the green area index for sweet corn in three water regimes I1(■), I2(●), and I3(▲) and nitrogen fertilizer applications (N1)(■), (N2)(▲), (N3)(●), (N4)(♦) and (N5)(×) (A, B)(C, D) in 2014 and 2015.The solid line represents the fitted regression $y = 0.96(1 − e^{−0.62x})$; $R^2 = 0.96$, $y = 0.97(1 − e^{−0.64x})$; $R^2 = 0.95$ and the dotted 90% PAR absorptance and critical green area index, respectively.

values were similar in the irrigation regime × N rate (Figure 2). That means the PNUE (the ratio of PN to SLN) was significantly affected by irrigation regime × N rate throughout the grain-filling phase, suggesting that the difference in PNUE among the irrigation regime was not consistent across N levels. As shown in Table 7, PNUE of the high levels of N was similar to that of the full irrigation, with both of them having smaller PNUE than the lower of N. Nitrogen limitation under N0 treatment further increased PNUE (Table 7). The N rate × I regime was significant for PNUE, suggesting that the difference in PNUE among the irrigation regime was not consistent across N levels. Leaf gs was affected by leaf position × N rate throughout the grain-filling phase (Table 5). As found in SLN, PN, and PNUE, the gs of the upper leaf was similar to that of the full irrigation, with both of them having greater gs than the lower of N. Nitrogen limitation at N0 treatment reduced gs at all irrigation regime (Table 5). In response to the water stress and the decline N fertilizer demand during the grain-filling, sweet corn adaptation mechanism is to reduce the stomatal conductance, and thus to decrease the water loss through the leaves, and consequently leaf transpiration rate (Table 5 and 6). Moreover, the magnitude of the stomatal closure and the decrement in the leaf transpiration is related to the level of water stress (Ripley et al, 2007). The reduced SG of leaves under low N conditions significantly affected light interception within

the canopy during the grain-filling phase. In general, there was greater irradiance at all irrigation regimes with high levels of nitrogen (Figure 2).

Discussion

During the grain-filling phase, the majority of N in the leaves is remobilized and translocated to the grain for protein synthesis (Hirel et al, 2007). A previous study showed that approximately 70% of the leaf N accumulated at the silking stage was translocated to the grain in maize (Chen et al, 2015). Therefore, it is a challenge for the plant to simultaneously use N efficiently and maintain a high rate of whole-plant photosynthesis during the grain-filling phase. These results quantify ear fresh yield responses when co-limited by water and nitrogen supply. Water stress prevented full use of applied nitrogen, highlighting the importance of balancing the amount of both resources to optimize ear fresh yield, production costs and environmental footprint, such as from winter leaching of residual N that is not taken up by water stressed crops in summer. The optimization of N allocation within the canopy can be a way to increase whole-plant photosynthesis (Hikosaka, 2014). Hirose and Werger (1987) showed that for a late goldenrod (*Solidago altissima* L) canopy, the potential increase in daily photosynthesis with non-uniform N content was 27%. In rice (*Oryza sativa* L), Hikosaka (2014) estimated that under direct-diffuse light, the loss in C

Table 3 - Specific leaf N in leaves during grain filling in 2014 and 2015.

		Sampling dates (day after silking)								
		0		10		20		30		40	
treatments[†]		Specific leaf N (gNm^{-2})									
Year		2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
I1	N0	0.83c[‡]	0.94c	0.64c	0.73c	0.59c	0.67c	0.46c	0.62c	0.39c	0.51c
	N125	1.59a	1.74a	1.23ab	1.34a	1.19ab	1.26ab	1.05ab	1.10ab	0.94ab	1.04ab
	N225	1.98a	1.90a	1.58a	1.55a	1.45a	1.50a	1.33a	1.38a	1.29a	1.33a
I3	N0	0.68d	0.75c	0.53c	0.57c	0.47d	0.54d	0.38c	0.42c	0.34c	0.41c
	N125	1.43c	1.47b	1.01bc	1.18b	0.95c	1.07c	0.86bc	0.89b	0.77b	0.83b
	N225	1.52ab	1.65ab	1.21b	1.25ab	1.14b	1.20b	1.01b	0.96b	0.97ab	0.93ab

[†]N0, N125, and N225 indicate N application of 0, 125, and 225 kg N ha^{-1}, respectively I1 and I3 indicate irrigation regime (I) application of 100 (I1) and 60 (I3) percent field capacity (FC). [‡]At the same sampling date, means in the same column followed by different letters indicate significant differences (P < 0.05) within nitrogen and water treatment.

gain because of suboptimal N distribution can be greater than 20%, and this loss can recover if N distribution gradient was steeper within the canopy. In general, more N is allocated to leaves under greater light intensities (Wyka et al, 2012) because the marginal C gain per unit N investment is more at greater light intensities than at reduced light intensities for a given N content (Hikosaka and Terashima, 1995). A decrease is known in the crop assimilation capacity of maize canopies due to low leaf nitrogen contents. This was indicated by the 50% decline in specific leaf nitrogen in response to both water and nitrogen stress (Drouet and Bonhom, 2004). Consistent with those results, our results showed that SLN and P_N were greater in full irrigation and N125, N225 than water stress and nitrogen in corresponding to the gradient of light distribution (Tables 3 and 4, Figure 2). George et al (2013) indicated that with low green leaf area index due to accelerated senescence, SLN changed more on a leaf area due to stress condition. Thus, on a leaf area basis, there is considerable redistribution of leaf N within the plant in both nitrogen and the level of water deficit. The distribution pattern of iPAR changed by GLAI because the pattern of leaf area was affected by different N treatments and water stress (Figure 3). This may explain why the interaction effect of N × irrigation was similar for the measured photosynthetic parameters throughout the grain-filling phase (Tables 4-6). The vertical distribution pattern of SLN and P_N was already established at

the silking stage and did not change during the grain-filling phase (Tables 3 and 4). Apart from the difference in irradiance among the irrigation regime, the rapid remobilization of N from the leaves during the grain-filling phase further contributed to the difference in SLN among leaves (Table 3). Furthermore, we found in the present study that PNUE among different treatments of I and N was also optimized together with SLN and P_N. That is, PNUE was greater in high levels of N and I than in lower ones (Table 7, Figure 2). Therefore, it seems that maize optimized its N resource allocation and photosynthetic system in response to light resource distribution to maximize photosynthetic production with N export during grain-filling phase. The P_N reported in the present study is lower than that reported in Echarte et al (2008). One possible explanation may be that the photosynthetic photon flux density used in this study is 1,600 µmol m^{-2} s^{-1}, lower than that in their study (2,000 µmol m^{-2} s^{-1}). The greater PNUE and P_N of the full irrigation treatments leaves might be partly related to their greater gs (Table 5). Zuo et al (2009) also found that the leaf gs increased in middle water stress. Also they found a close correlation between gs and P_N. A positive correlation between gs and P_N has been found in many other situations. For example, Hirasawa et al (1989) found that the reduction of P_N caused by drought stress was because of a decrease in CO_2 supply through the stomata to the mesophyll cells. Similar results have been recorded for

Table 4 - Net photosynthetic rate in leaves during grain filling in 2014 and 2015.

		Sampling dates (day after silking)								
		0		10		20		30		40	
treatments[†]		Net photosynthetic rate (μmol CO$_2$ m^{-2} s^{-1})									
Year		2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
I1	N0	11.23c[‡]	12.91c	11.28c	12.83c	9.56c	11.50c	9.42c	11.07c	8.64b	10.84b
	N125	22.40b	24.64b	21.23b	23.51b	18.29b	21.63b	13.45b	15.94b	11.07a	12.70a
	N225	29.07a	31.13a	27.14a	29.76a	25.76a	27.92a	17.09a	19.66a	11.87a	13.58a
I3	N0	7.81c	10.29c	7.86c	9.99c	7.37c	9.45c	7.06b	9.30b	6.29b	8.44b
	N125	12.13c	14.58a	12.01a	13.76b	10.90b	13.57a	10.45a	12.49a	9.87a	10.70a
	N225	14.00a	15.67a	13.72a	15.33a	12.41a	14.90a	11.40a	12.67a	9.67a	11.00a

[†]N0, N125, and N225 indicate N application of 0, 125, and 225 kg N ha^{-1}, respectively. I1 and I3 indicate irrigation regime (I) application of 100 (I1) and 60 (I3) percent field capacity (FC).[‡]At the same sampling date, means in the same column followed by different letters indicate significant differences (P < 0.05) within nitrogen and water treatment.

Table 5 - Stomatal conductance in leaves during grain filling in 2014 and 2015.

treatments[†]		Sampling dates (day after silking)									
		0		10		20		30		40	
		Stomatal conductance (mol H_2O m^{-2} s^{-1})									
Year		2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
I1	N0	0.11c[‡]	0.10c	0.10c	0.11c	0.09c	0.08c	0.07c	0.08c	0.05c	0.04c
	N125	0.29a	0.31a	0.28a	0.30a	0.26b	0.27b	0.14b	0.15b	0.08b	0.08b
	N225	0.35a	0.37a	0.34a	0.35a	0.31a	0.32a	0.23a	0.25a	0.10a	0.11a
I3	N0	0.06c	0.06b	0.05c	0.06b	0.05c	0.03c	0.04c	0.04c	0.03c	0.04c
	N125	0.14a	0.16a	0.13a	0.14a	0.11b	0.12b	0.07b	0.08b	0.05b	0.06b
	N225	0.16a	0.18a	0.18a	0.17a	0.14a	0.15a	0.11a	0.12a	0.07a	0.08a

[†]N0, N125, and N225 indicate N application of 0, 125, and 225 kg N ha^{-1}, respectively. I1 and I3 indicate irrigation regime (I) application of 100 (I1) and 60 (I3) percent field capacity (FC).[‡]At the same sampling date, means in the same column followed by different letters indicate significant differences (P < 0.05) within nitrogen and water treatment.

lupine (*Lupinus albus* L), eucalyptus (*Eucalyptus globulus* Labill), and sunflower (Helianthus annuus L) (Quick et al, 1992). Nitrogen-uptake efficiency is determined by both soil N supply and plant growth rate (Devienne-Barret et al, 2000). In the present study, both soil N availability and plant biomass production was less in N0 treatment, leading to reduced N accumulation compared with that in N175 and N225 treatment. Compared with N175 treatment, greater input of N under N225 treatment did not increase biomass (as shown by their similar ear fresh yield in Table 1) and, therefore, led to a decreased N-uptake efficiency. For grain maize production, NUtE is determined by both biomass production per unit N in the plant and grain yield (Sadras and Lemaire, 2014). Although ear fresh yield with N175 and N225 across irrigation regime were greater than that with N0, the NUtE with N175 and N225 was less than that with N0 (Table 1). The reason might be that with increasing N and irrigation supply, some N is used for restoring plant N status (Sadras and Lemaire, 2014), and some is used for N storage that is to be remobilized to the grains. Low N and water stress conditions resulted in lower SLN and P_N in the leaves; these parameters was changed as shown in the significant interaction effect of N × irrigation regime on these parameters (Table 3–6). This pattern is consistent with Anten et al (1995), who suggested the non-uniformity of N distribution increased with increasing total amount of free N in the canopy. The results presented here suggest

that N withdrawal within leaf and PNUE were further optimized by low N stress in maize. Nevertheless, the leaf PNUE was greater under low N than under sufficient N conditions (Table 7; Figure 2). The reason for the greater PNUE under low N stress needs to be further investigated. The decrease in gs under N0 and water stress suggests that gs does not explain our findings. Some biochemical and physiological properties of the leaves may have been optimized in response to low N stress to increase PNUE. In rice, for example, Li et al (2012) found that the reason for the reduced PNUE at high N supply was contributed by low Rubisco activity. Furthermore, although the GLAI was lower under low N conditions than under sufficient N conditions, the reduction of GLAI was predominantly in the water stress at late grain-filling stage. These data are similar to those found in Drouet and Bonhomme (2004) who suggested that maize plants allocated resources to maintain sufficient GLAI in the middle water stress to maximize light interception under low N conditions from silking to maturity. Modern breeding increased maize GY mainly by delaying leaf senescence and prolonging the duration of GLA from anthesis to harvest as well as the improvements in optimizing canopy architecture (Tollenaar and Lee, 2006). A challenge remains of how to further increase P_N and PNUE through modification in spatial N allocation as suggested by Hikosaka (2014). It may be possible to optimize to partition N among different N compounds and tissues. For example, an increase

Table 6 - Transpiration rate in leaves during grain filling in 2014 and 2015.

treatments[†]		Sampling dates (day after silking)									
		0		10		20		30		40	
		Transpiration rate (mol H_2O m^{-2} s^{-1})									
Year		2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
I1	N0	1.18b[‡]	1.48c	1.16c	1.36c	1.07b	1.22c	0.77b	0.62c	0.32b	0.43b
	N125	4.92a	5.05b	4.78b	4.82b	4.35a	4.58b	3.00a	3.61b	1.85a	2.03a
	N225	5.93a	6.55a	5.75a	6.42a	4.87a	6.03a	3.97a	4.73a	2.09a	2.27a
I3	N0	0.48c	0.61c	0.43c	0.56c	0.50c	0.48c	0.42c	0.45c	0.18b	0.24c
	N125	1.74b	1.86b	1.70b	1.76b	1.61b	1.72b	1.50b	1.61	1.11a	1.21a
	N225	2.83a	3.03a	2.74a	2.92a	2.60a	2.78a	2.43a	2.52ab	1.76a	1.85a

[†]N0, N125, and N225 indicate N application of 0, 125, and 225 kg N ha^{-1}, respectively. I1 and I3 indicate irrigation regime (I) application of 100 (I1) and 60 (I3) percent field capacity (FC).[‡]At the same sampling date, means in the same column followed by different letters indicate significant differences (P < 0.05) within nitrogen and water treatment.

Table 7 - Photosynthetic N-use efficiency in leaves during grain filling in 2014 and 2015.

treatments†		Sampling dates (day after silking)								
		0		10		20		30		40	
		Photosynthetic N-use efficiency ($\mu mol\ CO_2\ m^{-2}\ N\ s^{-1}$)									
Year		2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
I1	N0	24.45a‡	25.77a	26.82a	25.59a	26.14a	27.81a	20.34a	21.81a	22.42a	20.99a
	N125	14.12b	14.19b	17.21b	17.54b	15.34b	14.55b	12.86b	14.55b	11.72b	12.21b
	N225	14.07b	13.38b	16.00c	15.24c	11.85c	13.23c	12.88b	14.23b	9.23c	10.24c
I3	N0	11.50a	13.69a	14.81a	17.42a	15.65a	17.59a	18.57a	22.32a	18.41a	20.75a
	N125	8.47b	9.95b	11.90b	11.63b	11.49b	12.73b	12.11b	14.08b	12.11b	12.86b
	N225	9.19c	9.49b	11.34b	10.27c	10.93c	12.43b	10.32c	13.19c	9.95c	11.88b

†N0, N125, and N225 indicate N application of 0, 125, and 225 kg N ha^{-1}, respectively. I1 and I3 indicate irrigation regime (I) application of 100 (I1) and 60 (I3) percent field capacity (FC).‡At the same sampling date, means in the same column followed by different letters indicate significant differences (P < 0.05) within nitrogen and water treatment.

in leaf thickness may increase N accumulation, the chloroplast volume in the leaves and more stay green in sweet corn (Hikosaka, 2014). In this study, leave in water stress condition had reduced specific leaf area than lower amount of nitrogen (Table 3), suggesting that they may be thicker. With high amount of nitrogen conditions, there is greater N allocation to the C4 enzymes phosphoenolpyruvate carboxylase and pyruvate phosphate dikinase, which are closely related to greater biomass accumulation in maize (Sugiyama et al, 1984). Another way to explore the high P_N potential of the leaves may be to delay their senescence. (Valentinuz and Tollenaar, 2004). The fraction of intercepted PAR (iPAR) of different nitrogen and irrigation treatments were well related by asymptotic equations with the respective leaf area index (LAI). From the exponential curve in for three water regimes (a) and nitrogen fertilizer (b), the critical LAI (LAIcrit) at which 90% of intercepted PAR was calculated 4.35 and 4.1 in 2014 and 2015 respectively. (Figure 3). There were no differences in the extinction coefficient (k); hence a common function was fitted between the preparation iPAR and LAI for the 2014 and 2015 growing seasons. Both I1 and I2 were able to intercept 90% available PAR, while I0 almost approached the maximum value only when the highest nitrogen rate was applied (N225 and N175) in 2 years. From the exponential relationship, a values of K = 0.62 (R^2 = 96) and K = 0.64 (R^2 = 95) was determined for 2014 and 2015 respectively. Canopy architecture for sweet corn was consistent by N and irrigation treatments, hence K did not change. This suggests a homogenous canopy was achieved through the similarity in canopy architecture among the treatments. This was consistent with the work of Maddonni et al (2001), who observed no variation in the extinction coefficient between two maize hybrids once the critical green area index was achieved in populations of ≥ 9 plants m^{-2}. The kpar recorded in this study is close to the value of 0.70 reported by Plenet et al (2000) in maize and within the 0.45-0.90 range normally observed for most field crops (Stockle and Kemanian, 2009). The k of 0.62 and 0.64 determined here is similar to a previously reported value in maize (Echarte et al, 2008). It is

worth to mention that the fast canopy closure and growth rate of sweet corn enable it to intercept almost all available PAR when LAI is greater than 4.0. The early canopy closure is an advantage in terms of competition with weeds, making sweet corn an exceptional crop to be grown without any herbicide application. Irrigation and nitrogen supply enhance canopy closure stage, growth rate and biomass production per unit light intercepted.

Conclusion

Increasing ear fresh yield together with efficient N utilization is an important goal for sustainable sweet corn production. During the grain-filling phase and under stress conditions, while N is being continuously remo¬bilized, sweet corn plants not only maintain relatively greater SLN and P_N potential in the leaves to maintain whole-plant photosynthesis, but also use N more efficiently than in this condition, as revealed by their greater PNUE. The differences in PNUE among different N and I treatments be partly explained by their difference in gs. Low N facilitates leaf N remobilization without further optimizing distribution of SLN and PNUE. The greater whole-plant NUtE with low N may be explained by the overall increase in PNUE in the leaves at different N treatments and irrigation and the maintenance of light interception by more stay green in leaves.

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