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Uniconazole and nitrogen fertilization trigger photosynthesis and chlorophyll fluorescence, and delay leaf senescence in maize at a high population density

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Abstract

Reducing the leaf senescence rate could improve the grain yield in maize. In the present study, maize seeds were treated with uniconazole at concentrations of 0 and 25 mg kg^{-1}, and nitrogen was applied at 0, 150, and 225 kg ha^{-1} to maize crop planted at a density of 105,000 plants ha^{-1}. Applying uniconazole and nitrogen reduced the leaf senescence rate by increasing the leaf area per plant, chlorophyll content, net photosynthetic rate, stomatal conductance, and transpiration rate. Uniconazole and nitrogen increased the electron transport rate, photochemical quenching coefficient, variable fluorescence, maximal quantum yield of PSII photochemistry, and effective quantum yield of PSII photochemistry. Uniconazole and nitrogen reduced the intercellular carbon dioxide concentration, nonphotochemical quenching coefficient, and malondialdehyde content but increased the soluble protein content and antioxidant enzyme activities. Applying uniconazole at a concentration of 25 mg kg^{-1} and nitrogen at a rate of 150 kg ha^{-1} obtained the maximum grain yield in maize.

Keywords: leaf senescence; maize; nitrogen; plant population; uniconazole.

Introduction

Due to rapid increases in the world's population and reductions in arable lands, the global demand for maize will increase by 46% in 2050 (Hubert *et al.* 2010). Increasing plant populations is an important approach for improving the grain yield in maize but the high density plant populations lead to competition for resources,

Highlights

- Uniconazole and nitrogen application improved photosynthetic efficiency of maize
- Uniconazole and nitrogen application delayed leaf senescence in maize
- Treatment $U_{25}N_{150}$ obtained higher grain yield in maize

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Abbreviations: APX – ascorbate peroxidase; CAT – catalase; C_i – intercellular carbon dioxide concentration; E – transpiration rate; ETR – photosynthetic electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximum fluorescence yield of the dark-adapted state; F_v – variable fluorescence; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; MDA – malondialdehyde; P_N – net photosynthetic rate; POD – peroxidase; q_N – nonphotochemical quenching coefficient; q_P – photochemical quenching coefficient; SOD – superoxide dismutase; Φ_{PSII} – effective quantum yield of PSII photochemistry.

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thereby resulting in lodging, accelerated leaf senescence, and reduced photosynthetic activity (Xu *et al*. 2017, Xue *et al*. 2017, Kamran *et al*. 2018). The high density plant populations also decrease the photosynthetic efficiency and starch accumulation (Li *et al*. 2019). Leaf senescence involves a reduction in the leaf area at the bottom layer and eventually the whole plant (Gregersen *et al*. 2013, Ahmad *et al*. 2019a). Extending the photosynthetic duration is closely related to leaf senescence (Wu *et al*. 2012). Genetic and environmental factors (nutrient stress, water, and temperature) regulate leaf senescence in crops (van Doorn and Woltering 2004, Liu *et al*. 2005, Gregersen 2011, Thomas and Ougham 2015). The green leaf area and its duration have significant effects on the grain yield, and shaded leaves become senescent earlier compared to unshaded leaves (Maddonni *et al*. 2001, Gregersen *et al*. 2013). Accelerated leaf senescence leads to a lower grain yield in maize because of nutritional and hormonal signals (Sadras *et al*. 2000). Thus, reducing accelerated leaf senescence in dense plant populations is important for increasing the production of maize.

Nitrogen (N) is a major component of proteins and nucleic acids, and its application regulates plant growth and development (Dordas and Sioulas 2008, Li *et al*. 2012). N has a significant effect on leaf senescence traits and patterns (Kitonyo *et al*. 2018). Deficient or insufficient N fertilization inhibits the photosynthetic and radiation-use efficiencies to reduce the grain yield, whereas the optimum N application concentration can increase the grain yield (Shangguan *et al*. 2000, Dordas and Sioulas 2008, Luo *et al*. 2018). A previous study suggested that N has a positive correlation with the photosynthetic efficiency (Sage and Pearcy 1987). Fang *et al*. (2018a) reported that N fertilization enhanced the chlorophyll (Chl) content, net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and stomatal limitation value of buckwheat in different plant populations. N also improved the yield and yield components in cotton (Dong *et al*. 2012). N is important for enhancing the photosynthetic efficiency and leaf area (Sage and Pearcy 1987, Su *et al*. 2019). Wu *et al*. (2019) reported that low N stress significantly reduced the Chl content, P_N, g_s, and E, as well as increasing the intercellular carbon dioxide concentration (C_i) in maize cultivars.

Uniconazole (UCZ) is a triazole-type compound and its application can reduce the lodging rate and protect plants from various stresses (Duan *et al*. 2008, Wang *et al*. 2015a, Fang *et al*. 2018b). The application of triazole improved the P_N, E, maximal quantum yield of PSII photochemistry (F_v/F_m), photochemical quenching coefficient (q_p), and PSII efficiency in herbaceous peony (Xia *et al*. 2018), as well as enhancing the Chl content and P_N in *Cartharanthus roseus*, *Photinia*, *Pyracantha*, and dwarf Burford Holly (Frymire and Cole 1992, Jaleel *et al*. 2009). The application of triazole enhanced the grain yield and quality of rice by delaying leaf senescence due to the increased activities of antioxidant enzymes and reduced malondialdehyde (MDA) content (Pan *et al*. 2013). UCZ can reduce the degradation of Chl and enhance the activities of antioxidant enzymes (Zhang *et al*. 2007, Wang *et al*. 2009). Our previous study suggested that UCZ increased the Chl content, soluble protein (SP) content, P_N, and leaf area per maize plant in the bottom layer at the grain-filling stage (Ahmad *et al*. 2019a). Thus, previous research suggests that UCZ has a major effect on the regulation of leaf senescence.

Our previous study suggested that UCZ increased the lodging resistance and grain yield in wheat and maize (Ahmad *et al*. 2018a,b; 2019b, 2020). We also found that UCZ reduced leaf senescence in the bottom layer at the grain-filling stage in maize (Ahmad *et al*. 2019a). We also showed that the application of UCZ at a concentration of 25 mg kg^{-1} had a great effect on improving the lodging resistance, seed filling, and antioxidant defense system in medium density plant population. Thus, based on our previous studies, we applied 25 mg kg^{-1} UCZ and tested three different N rates in a high density maize plant population (105,000 plants ha^{-1}). We assessed the effects of UCZ and different N rates on the chlorophyll content, photosynthetic performance, chlorophyll fluorescence, antioxidant defense mechanism, and leaf senescence rate in maize. Our findings provide important insights into the delaying of leaf senescence and improving the grain yield in high density maize plant populations.

Materials and methods

Location, treatments, and field management: A field experiment was conducted during 2019 at the Institute of Water Saving Agriculture in Arid Areas of China (34°20'N, 108°04'E; 466.7 m a.s.l.). The average sunshine hours at the experimental site was 2,150 h, the annual average temperature was 12–14°C, and the total annual precipitation was 580.5 mm. During 2019, the total precipitation in the growth season was 541 mm and the average temperature was 21.8°C. The nutrient contents of the 0–40-cm soil layer comprised 1.10 g(total nitrogen) kg^{-1}, 56.14 mg(alkaline nitrogen) kg^{-1}, 10.61 mg(available phosphorus) kg^{-1}, and 139.19 mg(available potassium) kg^{-1}.

Maize hybrid Zhengdan 958 seeds were obtained from *Henan Golden Doll Seed Co. Ltd*, China. UCZ (5% WP) was obtained from Sichuan *Guoguang Agri-Chemical Co. Ltd.*, China. The experiment employed a factorial design and three replicates. The experiment consists of two factors, where factor A was the UCZ concentration, *i.e.*, 0 mg kg^{-1} (U_0) and 25 mg kg^{-1} (U_{25}), and factor B was the nitrogen rate, *i.e.*, 0 kg ha^{-1} (N_0, no N application), 150 kg ha^{-1} (N_{150}), and 225 kg ha^{-1} (N_{225}). Thus, the six combinations of treatments comprised U_0N_0, U_0N_{150}, U_0N_{225}, $U_{25}N_0$, $U_{25}N_{150}$, and $U_{25}N_{225}$. In our previous studies, we tested different UCZ concentrations and their application times in medium density plant populations. Based on our previous findings, we applied 25 mg(UCZ) kg^{-1} to the maize seeds and tested three different N application rates in a high density plant population. The seeds were treated with UCZ solution at concentrations of 0 mg kg^{-1} (U_0, distilled water) and 25 mg kg^{-1} (U_{25}) for 12 h at 25°C, and air dried before sowing. Each plot was 7 m long and 7 m wide (49 m^2). The distance between each plot was 1 m. The distance between rows was maintained at 60 cm and that between plants was 15.8 cm. Plots were manually planted with two seeds per mound and thinning was performed at the three-leaf stage (V3) to obtain a plant population with 105,000 plants ha^{-1}. Half of the N (urea, 46% N) was applied at the time of seed bed preparation and half at the jointing stage. Phosphorus (calcium superphosphate, 16% P_2O_5) and potassium (*Pudan Red*, 52% K_2O) were applied at sowing at a rate of 150 kg ha^{-1}. Sowing was conducted on 12 June and harvesting on 7 October in 2019. No irrigation was applied to the treatments during the growing season.

Leaf area per plant was measured at the V3 stage, eight-leaf stage (V8), silking stage (R1, occurs about 3 d after the tasseling stage and silk is visible outside the husk), 15 d after the silking stage (R1-15), 30 d after the silking stage (R1-30), and 45 d after the silking stage (R1-45) in the middle rows, except for five middle rows. Six plants per replicate were sampled in each stage and the leaf area per plant was calculated as follows: leaf area per plant [cm^2] = leaf length × leaf width × correction factor (0.75)

Chl *a* and *b* contents were determined for the uppermost leaf at V3 and V8, and the ear-leaf at R1, R1-15, R1-30, and R1-45 using six randomly selected plants for each of three replicates. The leaves initially used for obtaining photosynthesis and Chl fluorescence parameter measurements were sampled to determine the Chl content, MDA content, SP content, and antioxidant enzyme activities at V3, V8, R1, and R1-45, but not at R1-15 and R1-30. The Chl *a* and *b* contents were assayed according to Zhang *et al*. (2012). Briefly, 0.1 g of the middle leaf portion with the midrib removed was ground with liquid nitrogen and then soaked in the dark for 24 h in 10 mL of a solution containing 45% acetone, 45% ethanol, and 10% distilled water. The absorbance of the extract was measured at 663 and 646 nm with an ultraviolet (UV)-spectrophotometer (*UV-2600*, *Shimadzu Instruments*, Suzhou, China).

Photosynthetic parameters: At the V3, V8, R1 (silking stage), R3 (milk stage, 20 d after the silking stage), and R6 (physiological maturity stage) stages, P_N, g_s, C_i, and E were measured with a photosynthetic system (*LI-6400 XT*, *Li-Cor*, Lincoln, NE, USA). Six plants per replicate were selected and P_N, g_s, C_i, and E were measured using the fully expanded uppermost leaf at the V3 and V8 stages, and the ear leaves at R1, R3, and R6 on a clear sunny day between 9:30 and 11:30 h. The measurements of P_N, g_s, C_i, and E were acquired in a system with a leaf chamber temperature of 25°C and the carbon dioxide concentration in the leaf chamber was kept at 380 µmol(CO_2) mol^{-1} by using a CO_2 injector with a high-pressure liquid CO_2 cartridge source. The photosynthetic active radiation was set at 1,500 µmol(photon) m^{-2} s^{-1} (Wei *et al*. 2016). The external humidity was 40–50%.

The Chl fluorescence parameters were measured for the uppermost six expanded leaves per replicate at the V3 and V8 stages, and six ear leaves per replicate at the R1, R3, and R6 stages by using a portable chlorophyll fluorometer (*FMS 2.02*, *Hansatech Instruments Ltd*., United Kingdom). The leaves initially used for measuring the photosynthetic parameters were employed to determine the Chl fluorescence parameters. The minimal fluorescence (F_0) was determined for 60-min dark-adapted leaves and the maximum fluorescence (F_m) after a saturation light pulse [(8,000 µmol(photon) m^{-2} s^{-1})] of 0.8 s with the same leaves. The photosynthetic electron transport rate (ETR), q_P, nonphotochemical quenching coefficient (q_N), variable fluorescence, maximal quantum yield of PSII photochemistry, and effective quantum yield of PSII photochemistry were determined according to previously described methods (Zivcak *et al*. 2013, Mu *et al*. 2010).

MDA and SP contents and antioxidant enzyme activities: The MDA and SP contents and antioxidant enzyme activities were determined with six leaves initially used for analyzing the Chl contents at V3, V8, R1, R1-15, R1-30, and R1-45. First, 0.5 g of each leaf sample was homogenized in 5 mL of a pre-cooled mixture comprising 50 mmol L^{-1} phosphate buffer (pH 7.8), 0.1 mM EDTA-Na_2, and 1% (w/w) insoluble polyvinyl pyrrolidone (PVP), and then centrifuged at 15,000 × *g* for 10 min at 4°C. The supernatant was used for determining the MDA content, SP content, and antioxidant enzyme activities.

The MDA content was assayed according to Zhao *et al.* (2007), where 1 mL of extract was mixed with 2 mL of 0.67% thiobarbituric acid (TBA), heated at 100°C for 15 min in a water bath, and then centrifuged at 4,000 × *g* for 15 min after cooling. The absorbance of the supernatant was measured at 600, 532, and 450 nm with an UV-spectrophotometer (*UV-2600*, *Shimadzu Instruments*, Suzhou, China). The MDA content was expressed as µmol g^{-1}(FM).

The SP content was assayed according to Bradford (1976) and the result was expressed as mg g^{-1}(FM).

The activity of superoxide dismutase (SOD, EC 1.15.1.1) was determined according to Giannopolitis and Ries (1977), where 20 µL of extract was mixed with 3 mL of SOD reaction mixture (1.5 mL of 50 mmol L^{-1} phosphate buffer (pH 7.8), 0.3 mL of 0.75 mmol L^{-1} nitroblue tetrazolium, 0.3 mL of 130 mmol L^{-1} methionine, 0.3 mL of 0.02 mmol L^{-1} riboflavin, 0.3 mL of 0.1 mmol L^{-1} EDTA-Na_2, and 0.25 mL distilled water). The control and enzyme solution were placed in an incubator for 30 min at 5.86 W m^{-2} and the blank was kept in the dark. The absorbance was determined at 560 nm with an UV-spectrophotometer (*UV-2600*, *Shimadzu Instruments*, Suzhou, China).

The catalase (CAT, EC 1.11.1.6) activity was assayed according to Aebi (1984), where 50 µL of extract was mixed with 2.5 mL of CAT reaction mixture [0.1 mmol L^{-1} phosphate buffer (pH 7.0) and 0.1 mmol L^{-1} H_2O_2]. The absorbance was measured at 240 nm with an UV-spectrophotometer (*UV-2600*, *Shimadzu Instruments*, Suzhou, China).

The peroxidase (POD, EC 1.11.1.7) activity was assayed according to Hernández *et al*. (2000), where 20 µL of the extract was mixed with 3 mL of POD reaction solution [0.1 mmol L^{-1} phosphate buffer (pH 6.0), 16 mmol L^{-1}

guaiacol, and 19 µL of 10% (w/v) H_2O_2]. The absorbance was measured at 470 nm with an UV-spectrophotometer (*UV-2600*, *Shimadzu Instruments*, Suzhou, China).

The activity of ascorbate peroxidase (APX, EC 1.11.1.11) was determined according to Nakano and Asada (1981), where 0.2 mL of the extract was mixed with APX reaction mixture [50 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.5 mM AsA, and 1.0 mM H_2O_2]. The change in the absorbance was measured at 290 nm with an UV-spectrophotometer (*UV-2600*, *Shimadzu Instruments*, Suzhou, China).

Ear length, ear diameter, and grain yield: The grain yield was measured at maturity after harvesting the five central rows and avoiding the border rows in each of the three replicates. All of the plants were harvested from the five central rows in each replicate. The cobs were removed from the husk and threshed after 7 d to measure the grain yield. The ear length and ear diameter were measured based on ten ears per replicate. The ear length was measured with a measuring tape and the ear diameter using a digital Vernier caliper.

Statistical analysis: Analysis of variance (*ANOVA*) was performed using *Statistix 8.1*, where the data obtained from each sampling event and three replicates were analyzed separately. Comparisons of means were performed with the least significant difference (LSD) test at $P<0.05$.

Results

Leaf area per plant: UCZ and N significantly affected the leaf area per plant under a high density plant population (Table 1). The leaf area per plant tended to increase from the V3 stage and the maximum was reached at R1, then was gradually decreasing until R1-45. Increasing the N rate increased the leaf area per plant but decreased with 225 kg(N) ha^{-1} because the high N rate increased the plant height and reduced the leaf area per plant. The application of UCZ under different N rates significantly increased the leaf area per plant, and the maximum leaf area per plant was obtained with $U_{25}N_{150}$ in all growth stages, followed by U_0N_{150}. The lowest leaf area per plant in all growth stages was obtained with U_0N_0. U_0N_{150}, U_0N_{225}, $U_{25}N_0$, $U_{25}N_{150}$, and $U_{25}N_{225}$ increased the leaf area per plant at the V3 (by 94, 67, 11, 116, and 78%, respectively), V8 (by 151, 99, 16, 181, and 120%), R1 (by 113, 94, 23, 124, and 98%), R1-15 (by 145, 110, 31, 162, and 126%), R1-30 (by 187, 147, 42, 206, and 166%), and R1-45 (by 201, 160, 37, 226, and 180%) stages compared to U_0N_0.

Chl *a* and *b* contents: The application of UCZ and N significantly increased the Chl *a* and *b* contents at various growth stages (Table 2). The Chl *a* and *b* contents increased from V3 and the maximum occurred at R1, before decreasing until R1-45. Under U_0N_0 and $U_{25}N_0$, the maximum Chl *a* and *b* contents were found at V8, before decreasing until R1-45. Higher N rates decreased the Chl *a* and *b* contents, whereas the optimum N rate of 150 kg ha^{-1} yielded higher Chl *a* and *b* contents. The application of UCZ and N together increased the Chl *a* and *b* contents compared with the application of N alone. The minimum Chl *a* and *b* contents at all growth stages were obtained with U_0N_0 and the maximum with $U_{25}N_{150}$ followed by U_0N_{150}. The mean results for the six growth stages showed that compared with U_0N_0, treatments U_0N_{150}, U_0N_{225}, $U_{25}N_0$, $U_{25}N_{150}$, and $U_{25}N_{225}$ significantly increased the Chl *a* contents by 2.44, 1.81, 0.46, 2.73, and 2.07 mg g^{-1}, respectively, and the Chl *b* contents by 0.52, 0.34, 0.09, 0.66, and 0.42 mg g^{-1}.

Photosynthetic parameters: UCZ and N significantly affected P_N, g_s, C_i, and E under highly dense plant population (Tables 3, 4). UCZ and N significantly increased

Table 1. Effects of uniconazole (UCZ) and nitrogen fertilization on leaf area per plant at three-leaf stage (V3), eight-leaf stage (V8), silking stage (R1), and 15 (R1-15), 30 (R1-30), and 45 (R1-45) d after the silking stage in maize under high density plant population. ** – significant difference at 1% probability level; * – significant difference at 5% probability level; ns – no significant difference. Values are the means based on three replicates. *Different lowercase letters* within a column indicate significant differences at $P\leq0.05$ (LSD test). U_0N_0 [0 mg(UCZ) kg^{-1} + 0 kg(N) ha^{-1}]; U_0N_{150} [0 mg(UCZ) kg^{-1} + 150 kg(N) ha^{-1}]; U_0N_{225} [0 mg(UCZ) kg^{-1} + 225 kg(N) ha^{-1}]; $U_{25}N_0$ [25 mg(UCZ) kg^{-1} + 0 kg(N) ha^{-1}]; $U_{25}N_{150}$ [25 mg(UCZ) kg^{-1} + 150 kg(N) ha^{-1}]; $U_{25}N_{225}$ [25 mg(UCZ) kg^{-1} + 225 kg(N) ha^{-1}].

UCZ	Nitrogen	Leaf area per plant [cm^2]					
		V3	V8	R1	R1-15	R1-30	R1-45
U_0	N_0	64^d	941^f	3,053^e	2,532^f	2,005^f	1,707^f
U_0	N_{150}	123^b	2,362^b	6,512^b	6,200^b	5,761^b	5,143^b
U_0	N_{225}	106^c	1,874^d	5,924^c	5,323^d	4,943^d	4,430^d
U_{25}	N_0	71^d	1,087^e	3,767^d	3,323^e	2,856^e	2,343^e
U_{25}	N_{150}	137^a	2,647^a	6,834^a	6,634^a	6,134^a	5,563^a
U_{25}	N_{225}	113^c	2,070^c	6,045^c	5,734^c	5,342^c	4,785^c
ANOVA							
UCZ		**	**	**	**	**	**
N		**	**	**	**	**	**
UCZ × N		ns	ns	**	**	**	**

Table 2. Effects of uniconazole (UCZ) and nitrogen fertilization on chlorophyll (Chl) *a* and Chl *b* content at the three-leaf stage (V3), eight-leaf stage (V8), silking stage (R1), and 15 (R1-15), 30 (R1-30), and 45 (R1-45) d after the silking stage in maize under high density plant population. ** – significant difference at 1% probability level; * – significant difference at 5% probability level; ns – no significant difference. Values are the means based on three replicates. *Different lowercase letters* within a column indicate significant differences at $P \leq 0.05$ (LSD test). Abbreviations of the treatment names are the same as those given in Table 1.

UCZ	Nitrogen	Chl *a* [mg g^{-1}(FM)]						Chl *b* [mg g^{-1}(FM)]					
		V3	V8	R1	R1-15	R1-30	R1-45	V3	V8	R1	R1-15	R1-30	R1-45
U_0	N_0	2.36^{e}	4.34^{e}	3.41^{e}	2.71^{f}	2.10^{f}	0.61^{e}	0.46^{e}	0.86^{d}	0.70^{e}	0.58^{d}	0.10^{d}	0.08^{f}
U_0	N_{150}	4.02^{ab}	5.44^{b}	6.41^{a}	5.82^{b}	5.38^{b}	3.12^{b}	0.76^{b}	1.13^{b}	1.34^{b}	1.21^{b}	0.90^{a}	0.56^{b}
U_0	N_{225}	3.29^{c}	4.89^{c}	5.81^{c}	5.15^{d}	4.61^{d}	2.62^{c}	0.63^{c}	1.00^{c}	1.13^{c}	1.06^{c}	0.72^{b}	0.28^{d}
U_{25}	N_0	2.61^{d}	4.57^{d}	3.85^{d}	3.45^{e}	3.01^{e}	0.81^{d}	0.51^{d}	0.91^{d}	0.84^{d}	0.65^{d}	0.25^{c}	0.14^{e}
U_{25}	N_{150}	4.19^{a}	5.91^{a}	6.53^{a}	6.01^{a}	5.74^{a}	3.53^{a}	0.82^{a}	1.21^{a}	1.59^{a}	1.39^{a}	0.96^{a}	0.76^{a}
U_{25}	N_{225}	3.88^{b}	5.01^{c}	6.04^{d}	5.34^{c}	5.01^{c}	2.68^{c}	0.74^{b}	1.04^{c}	1.23^{bc}	1.06^{c}	0.76^{b}	0.47^{c}
ANOVA													
UCZ		**	**	**	**	**	**	**	**	**	*	**	**
N		**	**	**	**	**	**	**	**	**	**	**	**
UCZ × N		**	*	*	**	**	**	ns	ns	ns	ns	ns	**

Table 3. Effects of uniconazole (UCZ) and nitrogen fertilization on net photosynthetic rate (P_N) and stomatal conductance (g_s) at the three-leaf stage (V3), eight-leaf stage (V8), silking stage (R1), milk stage (R3), and physiological maturity stage (R6) in maize under high density plant population. ** – significant difference at 1% probability level; * – significant difference at 5% probability level; ns – no significant difference. Values are the means based on three replicates. *Different lowercase letters* within a column indicate significant differences at $P \leq 0.05$ (LSD test). Abbreviations of the treatment names are the same as those given in Table 1.

UCZ	Nitrogen	P_N [µmol m^{-2} s^{-1}]					g_s [mol(H_2O) m^{-2} s^{-1}]				
		V3	V8	R1	R3	R6	V3	V8	R1	R3	R6
U_0	N_0	17.2^{f}	21.4^{e}	19.4^{f}	15.8^{e}	6.0^{e}	0.08^{e}	0.14^{e}	0.18^{d}	0.09^{d}	0.08^{c}
U_0	N_{150}	27.8^{b}	32.3^{b}	40.6^{b}	35.3^{a}	18.1^{b}	0.18^{b}	0.24^{b}	0.29^{ab}	0.22^{ab}	0.16^{ab}
U_0	N_{225}	22.2^{d}	27.1^{c}	34.7^{d}	29.7^{c}	16.3^{c}	0.16^{c}	0.19^{cd}	0.24^{c}	0.18^{c}	0.13^{b}
U_{25}	N_0	19.0^{e}	24.7^{d}	22.1^{e}	18.4^{d}	8.8^{d}	0.12^{d}	0.16^{de}	0.20^{d}	0.11^{d}	0.09^{c}
U_{25}	N_{150}	29.3^{a}	34.8^{a}	43.2^{a}	37.1^{a}	22.1^{a}	0.22^{a}	0.28^{a}	0.32^{a}	0.24^{a}	0.18^{a}
U_{25}	N_{225}	24.2^{c}	28.9^{c}	37.5^{c}	31.9^{b}	17.4^{bc}	0.18^{b}	0.21^{bc}	0.26^{bc}	0.20^{bc}	0.14^{b}
ANOVA											
UCZ		**	**	**	**	**	**	**	*	**	ns
N		**	**	**	**	**	**	**	**	**	**
UCZ × N		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

P_N, g_s, and *E* at all growth stages, but decreased C_i. P_N, g_s. *E* increased from the V3 stage and the maximum values were reached at R1, then it was decreasing until R6. C_i increased from the V3 stage and the maximum values were reached at R6. Under U_0N_0 and $U_{25}N_0$, the maximum P_N values were reached at V8, then were gradually decreasing until R6. Increasing the N rate increased P_N, g_s, and *E* but the higher N rate of 225 kg ha^{-1} decreased P_N, g_s, and *E*. The application of UCZ together with N obtained higher P_N, g_s, and *E* values but decreased C_i compared with the application of N alone. The maximum P_N, g_s, and *E* values and minimum C_i values were obtained under $U_{25}N_{150}$ followed by U_0N_{150}, whereas the minimum P_N, g_s, and *E* values and maximum C_i values were recorded under U_0N_0. Our results suggest that the application of UCZ together with N significantly increased the Chl content to enhance the photosynthetic capacity of maize plants. The mean results in five growth stages showed that compared with U_0N_0, treatments U_0N_{150}, U_0N_{225}, $U_{25}N_0$, $U_{25}N_{150}$, and $U_{25}N_{225}$ increased P_N by 93.1, 62.9, 16.5, 108.6, and 75.3%, respectively, g_s by 91.2, 57.9, 19.3, 117.5, and 73.7%, and *E* by 74.8, 42.5, 12.6, 95.3, and 56.7%, but decreased C_i by 107.3, 63.3, 21.8, 138.1, and 84.9%.

Chl fluorescence: The application of UCZ and N significantly affected ETR, q_p, q_N, F_v/F_m, Φ_{PSII}, and F_v in the high density plant population (Tables 5, 6, 7). UCZ and N increased ETR, q_p, F_v/F_m, Φ_{PSII}, and F_v but decreased q_N. ETR, q_p, and Φ_{PSII} increased from the V3 stage and the maximum values were reached at R1, then were decreasing until R6. Under U_0N_0 and $U_{25}N_0$, ETR, q_p, and Φ_{PSII} increased from the V3 stage and the peak values were reached at the V8 stage, then decreased. q_N was the highest at the V3 stage but it then decreased at the V8 stage, before increasing

Table 4. Effects of uniconazole (UCZ) and nitrogen fertilization on intercellular CO_2 concentration (C_i) and transpiration rate (E) at the three-leaf stage (V3), eight-leaf stage (V8), silking stage (R1), milk stage (R3), and physiological maturity stage (R6) in maize under high density plant population. ** – significant difference at 1% probability level; * – significant difference at 5% probability level; ns – no significant difference. Values are the means based on three replicates. *Different lowercase letters* within a column indicate significant differences at $P \leq 0.05$ (LSD test). Abbreviations of the treatment names are the same as those given in Table 1.

UCZ	Nitrogen	C_i [mol(CO_2) m^{-2} s^{-1}]					E [mmol(H_2O) m^{-2} s^{-1}]				
		V3	V8	R1	R3	R6	V3	V8	R1	R3	R6
U_0	N_0	74^{a}	159^{a}	187^{a}	203^{a}	370^{a}	2.6^{e}	3.1^{e}	3.5^{e}	2.2^{e}	1.3^{d}
U_0	N_{150}	37^{de}	64^{e}	81^{d}	115^{e}	182^{d}	4.6^{b}	5.7^{ab}	6.4^{ab}	3.5^{ab}	2.0^{ab}
U_0	N_{225}	47^{c}	87^{c}	112^{c}	145^{c}	217^{c}	3.1^{d}	5.0^{c}	5.6^{c}	2.9^{cd}	1.5^{cd}
U_{25}	N_0	67^{b}	129^{b}	157^{b}	178^{b}	284^{b}	2.1^{f}	3.8^{d}	4.4^{d}	2.5^{de}	1.5^{cd}
U_{25}	N_{150}	33^{e}	53^{f}	69^{e}	98^{f}	164^{e}	5.5^{a}	6.3^{a}	7.0^{a}	3.8^{a}	2.2^{a}
U_{25}	N_{225}	40^{d}	75^{d}	102^{c}	130^{d}	190^{d}	3.7^{c}	5.3^{bc}	6.1^{bc}	3.1^{bc}	1.7^{bc}
ANOVA											
UCZ		**	**	**	**	**	*	**	**	ns	*
N		**	**	**	**	**	**	**	**	**	**
UCZ × N		ns	**	*	ns	**	**	ns	ns	ns	ns

Table 5. Effects of uniconazole (UCZ) and nitrogen fertilization on photosynthetic electron transport rate (ETR) and photochemical quenching coefficient (q_p) at the three-leaf stage (V3), eight-leaf stage (V8), silking stage (R1), milk stage (R3), and physiological maturity stage (R6) in maize under high density plant population. ** – significant difference at 1% probability level; * – significant difference at 5% probability level; ns – no significant difference. Values are the means based on three replicates. *Different lowercase letters* within a column indicate significant differences at P0.05 (LSD test). Abbreviations of the treatment names are the same as those given in Table 1.

UCZ	Nitrogen	ETR					q_p				
		V3	V8	R1	R3	R6	V3	V8	R1	R3	R6
U_0	N_0	106^{d}	121^{e}	98^{f}	85^{f}	56^{f}	0.23^{d}	0.29^{d}	0.27^{d}	0.23^{c}	0.15^{f}
U_0	N_{150}	147^{ab}	164^{b}	172^{b}	149^{b}	90^{b}	0.30^{ab}	0.39^{ab}	0.45^{a}	0.41^{a}	0.28^{b}
U_0	N_{225}	131^{c}	147^{c}	152^{d}	127^{d}	79^{d}	0.27^{c}	0.35^{c}	0.39^{b}	0.35^{b}	0.23^{d}
U_{25}	N_0	113^{d}	132^{d}	115^{e}	95^{e}	62^{e}	0.24^{d}	0.32^{d}	0.30^{c}	0.25^{c}	0.17^{e}
U_{25}	N_{150}	156^{a}	169^{a}	179^{a}	162^{a}	95^{a}	0.32^{a}	0.41^{a}	0.47^{a}	0.43^{a}	0.33^{a}
U_{25}	N_{225}	138^{bc}	149^{c}	161^{c}	137^{c}	83^{c}	0.29^{bc}	0.37^{bc}	0.41^{b}	0.37^{b}	0.24^{c}
ANOVA											
UCZ		*	*	**	**	**	*	*	**	*	**
N		**	**	**	**	**	**	**	**	**	**
UCZ × N		ns	ns	*	ns	ns	ns	ns	ns	ns	**

from R1 to R6. F_v/F_m and Φ_{PSII} were the highest at the V3 stage but they decreased at the V8 stage, increased again at the R1 stage, and then decreased gradually until maturity. Increasing the N rate significantly increased ETR, q_p, F_v/F_m, Φ_{PSII}, and F_v, where 150 kg(N) ha^{-1} obtained better results compared to the high N rate. The application of UCZ together with N increased ETR, q_p, F_v/F_m, Φ_{PSII}, and F_v, and decreased q_N compared to the application of N alone. The maximum ETR, q_p, F_v/F_m, Φ_{PSII}, and F_v values and the lowest q_N values in all growth stages were obtained with $U_{25}N_{150}$ followed by U_0N_{150}. U_0N_0 obtained lower ETR, q_p, F_v/F_m, Φ_{PSII}, and F_v values and higher q_N values. The mean results based on five growth stages showed that compared to U_0N_0, U_0N_{150}, U_0N_{225}, $U_{25}N_0$, $U_{25}N_{150}$, and $U_{25}N_{225}$ increased ETR by 54.9, 36.5, 10.9, 63.3, and 43.3%, respectively, q_p by 56.4, 35.9, 9.4, 67.5, and 43.6%, F_v/F_m by 16.3, 10.3, 4.2, 18.7, and 13%, Φ_{PSII} by 67.5, 40.2, 12.8, 83.8, and 51.3%, and F_v by 31.0, 18.6, 7.1, 37.0, and 23.7%, but reduced q_N by 11.2, 4.9, 2.4, 16.0, and 8.4%.

MDA and SP contents: The application of UCZ and N fertilizer reduced the MDA contents and increased the SP contents in the high density plant population (Table 1S, *supplement*). The MDA contents increased from V3 to V8, decreased at R1, and then tended to increase until R1-45. The SP contents increased from V3 to V8, decreased at R1, increased until R1-30, and again decreased at R1-45. Compared to the application of N alone, the application of UCZ together with N increased the SP contents and decreased the MDA contents. The maximum MDA content in all growth stages were recorded under U_0N_0

Table 6. Effects of uniconazole (UCZ) and nitrogen fertilization on nonphotochemical quenching coefficient (q_N) and maximal quantum efficiency of PSII photochemistry (F_v/F_m) at the three-leaf stage (V3), eight-leaf stage (V8), silking stage (R1), milk stage (R3), and physiological maturity stage (R6) in maize under high density plant population. ** – significant difference at 1% probability level; * – significant difference at 5% probability level; ns – no significant difference. Values are the means based on three replicates. *Different lowercase letters* within a column indicate significant differences at $P≤0.05$ (LSD test). Abbreviations of the treatment names are the same as those given in Table 1.

UCZ	Nitrogen	q_N					F_v/F_m				
		V3	V8	R1	R3	R6	V3	V8	R1	R3	R6
U_0	N_0	0.86^a	0.83^a	0.85^a	0.86^a	0.88^a	0.75^c	0.71^d	0.67^e	0.62^e	0.56^e
U_0	N_{150}	0.81^c	0.76^{bc}	0.73^{de}	0.76^d	0.79^d	0.80^{ab}	0.77^{ab}	0.80^{ab}	0.76^{ab}	0.72^a
U_0	N_{225}	0.83^b	0.81^a	0.80^{bc}	0.81^{bc}	0.83^c	0.77^{bc}	0.75^{bc}	0.76^c	0.71^c	0.66^c
U_{25}	N_0	0.84^b	0.81^a	0.83^{ab}	0.84^{ab}	0.86^b	0.77^{bc}	0.73^{cd}	0.70^d	0.66^d	0.59^d
U_{25}	N_{150}	0.78^d	0.73^c	0.70^e	0.72^e	0.76^e	0.81^a	0.78^a	0.82^a	0.79^a	0.73^a
U_{25}	N_{225}	0.80^c	0.79^{ab}	0.77^{cd}	0.79^{cd}	0.80^d	0.78^{abc}	0.76^{ab}	0.78^{bc}	0.74^{bc}	0.68^b
ANOVA											
UCZ		**	ns	**	*	**	ns	ns	**	**	**
N		**	**	**	**	**	**	**	**	**	**
UCZ × N		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 7. Effects of uniconazole (UCZ) and nitrogen fertilization on effective quantum yield of PSII photochemistry (Φ_{PSII}) and variable fluorescence (F_v) at the three-leaf stage (V3), eight-leaf stage (V8), silking stage (R1), milk stage (R3), and physiological maturity stage (R6) in maize under high density plant population. ** – significant difference at 1% probability level; * – significant difference at 5% probability level; ns – no significant difference. Values are the means based on three replicates. *Different lowercase letters* within a column indicate significant differences at $P≤0.05$ (LSD test). Abbreviations of the treatment names are the same as those given in Table 1.

UCZ	Nitrogen	Φ_{PSII}					F_v				
		V3	V8	R1	R3	R6	V3	V8	R1	R3	R6
U_0	N_0	0.22^c	0.29^d	0.27^f	0.23^e	0.16^f	0.92^c	0.89^d	0.82^d	0.73^e	0.61^f
U_0	N_{150}	0.29^{ab}	0.40^{ab}	0.53^b	0.42^b	0.32^b	1.15^a	1.07^{ab}	1.11^{ab}	1.01^{ab}	0.86^b
U_0	N_{225}	0.27^b	0.37^b	0.42^d	0.33^{cd}	0.25^d	1.05^b	0.99^c	1.02^c	0.89^c	0.76^d
U_{25}	N_0	0.23^c	0.33^c	0.30^e	0.27^{de}	0.19^e	1.01^b	0.92^d	0.87^d	0.80^d	0.65^e
U_{25}	N_{150}	0.31^a	0.42^a	0.56^a	0.51^a	0.35^a	1.19^a	1.10^a	1.16^a	1.07^a	0.92^a
U_{25}	N_{225}	0.28^b	0.38^b	0.46^c	0.38^{bc}	0.27^c	1.05^b	1.03^{bc}	1.06^{bc}	0.96^b	0.81^c
ANOVA											
UCZ		ns	*	**	**	**	*	*	**	**	**
N		**	**	**	**	**	**	**	**	**	**
UCZ × N		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

and the minimum under $U_{25}N_{150}$. The highest SP contents in all growth stages were recorded under $U_{25}N_{150}$ and the minimum under U_0N_0. The mean results based on six growth stages showed that compared to U_0N_0, U_0N_{150}, U_0N_{225}, $U_{25}N_0$, $U_{25}N_{150}$, and $U_{25}N_{225}$ decreased the MDA contents by 39.7, 27.6, 12.6, 62.9, and 42%, respectively, and increased the SP contents by 33.1, 22, 6.7, 57.4, and 33.5%.

Antioxidant enzyme activities: The antioxidant enzyme activities increased significantly by UCZ and N in the high density plant population (Tables 2S, 3S, *supplement*). The activity of SOD increased from V3 to R1-15 and then decreased. The activity of POD increased from V3 to R1-30 and then decreased. The activities of CAT and APX were the highest at V3, decreased at V8, and then increased from R1 until R1-45. Increasing N enhanced the antioxidant enzyme activities but they decreased when N was applied at 225 kg ha^{-1}. The application of UCZ together with N obtained higher antioxidant enzyme activities compared to the application of N alone. The minimum antioxidant enzyme activities were recorded under U_0N_0 and the maximum activities under $U_{25}N_{150}$. The mean results based on six growth stages showed that compared to U_0N_0, U_0N_{150}, U_0N_{225}, $U_{25}N_0$, $U_{25}N_{150}$, and $U_{25}N_{225}$ increased the activities of SOD by 37, 27.2, 8.8, 49.7, and 35.8%, respectively, POD by 44.7, 33.2, 10.4, 63.6, and 47.0%, CAT by 46.3, 31.8, 9.7, 71.2, and 55.4%, and APX by 32.8, 23.3, 8.6, 52.7, and 38.2%. The higher antioxidant enzyme activities significantly protected the photosynthetic system and increased the grain yield in maize.

Table 8. Effects of uniconazole (UCZ) and nitrogen fertilization on ear length, ear diameter, and grain yield in maize under high density plant population. ** – significant difference at 1% probability level; * – significant difference at 5% probability level; ns – no significant difference. Values are the means based on three replicates. *Different lowercase letters* within a column indicate significant differences at $P \le 0.05$ (LSD test). Abbreviations of the treatment names are the same as those given in Table 1.

UCZ	Nitrogen	Ear length [cm]	Ear diameter [mm]	Grain yield [kg ha^{-1}]
U_0	N_0	10.6^e	38.9^d	4,134^e
U_0	N_{150}	16.0^b	48.2^b	10,676^{bc}
U_0	N_{225}	14.7^c	46.9^b	10,310^c
U_{25}	N_0	11.4^d	41.8^c	5,150^d
U_{25}	N_{150}	16.9^a	50.7^a	13,728^a
U_{25}	N_{225}	15.1^c	48.2^b	11,100^b
ANOVA				
UCZ		**	**	**
N		**	**	**
UCZ × N		ns	ns	**

Ear length, ear diameter, and grain yield: The application of UCZ and N significantly increased the ear length, ear diameter, and grain yield in maize at a high density plant population (Table 8). Compared to the application of N alone, applying UCZ together with N increased the ear length, ear diameter, and grain yield with a high density plant population. The minimum ear length, ear diameter, and grain yield were obtained under U_0N_0 and the maximum values under $U_{25}N_{150}$. Compared to U_0N_0, U_0N_{150}, U_0N_{225}, $U_{25}N_0$, $U_{25}N_{150}$, and $U_{25}N_{225}$ increased the ear length by 50.9, 38.7, 7.5, 59.4, and 42.5%, respectively, the ear diameter by 23.9, 20.6, 7.5, 30.3, and 23.9%, and the grain yield by 158.2, 149.4, 24.6, 232.1, and 168.5%.

Discussion

Chl contents, SP contents, photosynthesis, and relationships with leaf senescence: Reducing maize accelerated leaf senescence and protecting the photosynthetic apparatus is important for a high density plant population. Leaf senescence is closely correlated with reductions in the Chl content and leaf area per plant (Wang *et al*. 2009). Chl degradation reduces the photosynthetic efficiency (Wang *et al*. 2015b). In our previous study, we found that UCZ reduced leaf senescence in the bottom layer at the grain-filling stage in maize and increased the Chl contents and P_N in a medium density plant population (Ahmad *et al.* 2019a). In the present study, we applied 25 mg(UCZ) kg^{-1} and different N rates in the high density plant population. Our results suggested that the leaf area per plant and Chl *a* and *b* contents gradually increased from V3 to R1, then were decreasing as the plants became older (Tables 1, 2). Under U_0N_0 and $U_{25}N_0$, the leaf area per plant, Chl *a* and *b* contents, and P_N increased from V3 to V8, then they were decreasing until maturity. Furthermore, the leaf area per plant, Chl *a* and *b* contents, SP contents, P_N, g_s, and *E* were significantly lower, whereas C_i was higher under U_0N_0 and $U_{25}N_0$. Under U_0N_0 and $U_{25}N_0$, leaf senescence started after V8, and thus the Chl contents and photosynthetic efficiency were lower. Applying UCZ together with N increased the leaf area per plant, Chl *a* and *b* contents, SP contents, P_N, g_s, and *E* but decreased C_i. UCZ application together with N increased the leaf area per plant, Chl *a* and *b* contents, SP contents, P_N, g_s, and *E* but decreased C_i compared to the application of N alone. $U_{25}N_{150}$ obtained the maximum leaf area per plant, Chl *a* and *b* contents, SP contents, P_N, g_s, and *E* but decreased C_i. Our results suggest that UCZ and N had crucial effects on enhancing the photosynthetic efficiency and crop growth. In the present study, the application of UCZ together with N at different rates significantly increased the leaf area per plant, Chl contents, and photosynthetic efficiency. UCZ is a plant growth regulator that blocks gibberellic acid biosynthesis by inhibiting the conversion of kaurene to kaurenoic acid (Davis *et al*. 1988). Furthermore, UCZ inhibits the activity of ent-kaurene oxidase, thereby resulting in the accumulation of geranyl diphosphate and enhancing Chl biosynthesis (Soumya *et al*. 2017). UCZ alters the endogenous hormone contents by inhibiting gibberellic acid to increase the abscisic acid and cytokinin contents. Our recent study of wheat suggested that UCZ affected the endogenous hormone contents of the flag leaves to increase the Chl contents and canopy apparent photosynthesis (Ahmad *et al*. 2021).

Effects of UCZ and N fertilization on Chl fluorescence parameters: Chl fluorescence is an important component of photosynthesis and it can be measured to evaluate the state of PSII and the yield performance (Wang *et al.* 2013, Ali *et al*. 2018). Chl fluorescence increases in newly expanding leaves but decreases significantly as leaf senescence proceeds (Zhong *et al*. 2019). In our previous study, we did not measure the Chl fluorescence parameters. Thus, in the present study, we measured the Chl fluorescence parameters at five different growth stages in order to determine the effects of UCZ and N on these parameters during the leaf senescence process. Our results suggested that the application of UCZ together with N significantly affected the Chl fluorescence parameters (Tables 5, 6, 7). In particular, the application of UCZ and

N significantly increased ETR, q_p, F_v/F_m, Φ_{PSII}, and F_v, but decreased q_N in all growth stages. The application of UCZ with N resulted in higher ETR, q_p, F_v/F_m, Φ_{PSII}, and F_v values but lower q_N values compared to the application of N alone. The maximum ETR, q_p, F_v/F_m, Φ_{PSII}, and F_v values, and the lowest q_N values were recorded under $U_{25}N_{150}$, whereas the lowest ETR, q_p, F_v/F_m, Φ_{PSII}, and F_v values, and the highest q_N values were obtained under U_0N_0. It is known that N deficiency can decrease q_p and F_v/F_m but increase q_N to affect the photochemical activity of PSII and photosynthesis (Yu *et al*. 2004). N stress significantly reduces ETR, F_v/F_m, q_p, and Φ_{PSII} but increases q_N in maize (Wu *et al*. 2019). Our results indicate that U_0N_0 caused photoinhibition and damaged the potential active center of PSII. Our findings suggest that the application of UCZ together with N protected the photosynthetic system. N at a rate of 225 kg ha^{-1} decreased ETR, q_p, F_v/F_m, Φ_{PSII}, and F_v but increased q_N. A previous study showed that a higher N application rate could increase q_N but decrease ETR, q_p, and F_v/F_m (Shrestha *et al*. 2012). In addition, the optimum N application rate increased F_v, F_m, F_v/F_m, Φ_{PSII}, ETR, and q_p in naked oats but F_v/F_m, ETR, q_p, and Φ_{PSII} decreased in cotton when N was applied at a high rate (Lin *et al*. 2013). Previous studies demonstrated that the energy transformation efficiency in the PSII reaction center and photosynthesis increased by the application of plant growth regulators (Wang *et al*. 2013, Ajigboye *et al*. 2014). Thus, our findings indicate that the application of UCZ together with N significantly increased ETR, q_p, F_v/F_m, Φ_{PSII}, and F_v but decreased q_N during the leaf senescence process and protected the photosynthetic system.

Role of MDA accumulation in leaf senescence process: Leaf senescence enhances the MDA contents, which can have detrimental effects on plant growth (Zhang *et al*. 2010). The increased accumulation of MDA has been detected in the leaf senescence process in various crops (Zhang *et al*. 2010, Wang *et al*. 2015b). Our results showed that the MDA contents were lower at R1 and they increased gradually as leaf senescence proceeded. Applying UCZ together with N decreased the MDA contents during the leaf senescence process. The MDA contents were lower when UCZ was applied together with N compared to the application of N alone. Lower MDA contents were obtained in all growth stages under $U_{25}N_{150}$ and the maximum contents were found under U_0N_0. A previous study also found that UCZ reduced the MDA contents (Wang *et al*. 2009). In addition, our previous study showed that UCZ reduced the MDA contents in wheat and maize (Ahmad *et al*. 2019a,b).

Antioxidant enzyme activities and roles in leaf senescence: Leaf senescence results in the accumulation of high amounts of reactive oxygen species (ROS) and the activities of antioxidant enzymes can reduce the ROS contents in plants (El Hadrami *et al*. 2005, Li *et al*. 2007). Our previous study of wheat showed that UCZ increased the antioxidant enzyme activities and the higher activity levels decreased the accumulation of ROS (Ahmad *et al*. 2019b). Triazoles may increase the antioxidant enzyme activities and reduce the ROS contents in wheat, rice, and winter rape (Zhang *et al*. 2010, Pan *et al*. 2013). Our results showed that the activity of SOD increased from V3 to R1-15 and then decreased. The activity of POD increased from V3 to R1-30 and then decreased. The activities of CAT and APX were higher at V3, decreased at V8, and then they tended to increase until maturity. The application of UCZ together with N significantly increased the antioxidant enzyme activities, where the maximum values were obtained under $U_{25}N_{150}$ and the minimum values under U_0N_0. We also found that the application of N alone increased the antioxidant enzyme activities and reduced leaf senescence. The higher antioxidant enzyme activities protected the Chl pigments to enhance the photosynthetic efficiency and reduce the leaf senescence rate in the high density plant population. The application of N at a rate of 225 kg ha^{-1} decreased the leaf area per plant, Chl contents, SP contents, P_N, g_s, E, ETR, q_P, F_v/F_m, Φ_{PSII}, F_v, antioxidant enzyme activities, yield, and yield components compared to those when N was applied at 150 kg ha^{-1}, probably due to the inhibitory effect of the higher N rate. In addition, the higher N rate may have resulted in taller plants with a higher lodging rate. However, this difference requires further investigation.

Effects of UCZ and N fertilization on grain yield of maize crop: The application of UCZ together with N significantly increased the maize grain yield under the high density plant population. The higher grain yield under UCZ together with N in the high density plant population was attributable to the higher Chl contents, photosynthetic efficiency, and Chl fluorescence, and the enhanced antioxidant defense system. The application of UCZ together with N protected the photosynthetic system and obtained higher grain yields. Previous studies also showed that the application of UCZ together with N increased the grain yield in various crops by reducing the lodging rate and enhancing the photosynthetic performance (Wang *et al*. 2009, 2015a; Su *et al*. 2019). In addition, our previous studies demonstrated that UCZ increased the grain yield in maize with a medium plant population density by reducing leaf senescence in the bottom layer. In the present study, the application of UCZ at a concentration of 25 mg kg^{-1} and nitrogen at a rate of 150 kg ha^{-1} resulted in the maximum grain yield in a high density plant population.

Conclusion: In the present study, we found that the application of UCZ and N fertilizer significantly increased the maize grain yield under high density plant population. The application of UCZ and N increased the leaf area per plant, chlorophyll *a* and *b* contents, P_N, g_s, and E but reduced C_i. Applying UCZ together with N also increased ETR, q_p, F_v/F_m, Φ_{PSII}, and F_v but reduced q_N. Furthermore, UCZ together with N enhanced the SP content and antioxidant enzyme activities but reduced the MDA content. The lower MDA content and higher antioxidant enzyme activities protected the photosynthetic pigments and increased the leaf area per plant. Compared to the application of N alone, applying UCZ together with N increased the leaf area per plant, chlorophyll *a* and *b* contents, P_N, g_s, E, ETR, q_p, F_v/F_m, Φ_{PSII}, and F_v but decreased C_i, q_N, and the MDA content. The application of UCZ together with N decreased the degradation of chlorophyll and reduced the leaf senescence rate under high density plant population, and $U_{25}N_{150}$ obtained the maximum grain yield of 13,728 kg ha^{-1}.

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