



Both uniconazole and 5-aminolevulinic acid increase maize (*Zea mays* L.) yield by changing its ear morphology and increasing photosynthetic efficiency and antioxidants in saline-alkali land

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Abstract

Saline-alkaline stress is one of the most detrimental abiotic stresses that restrict the yield and physiological activity of maize (*Zea mays* L.). In the present study, maize was planted on saline-alkali land, while 25 mg L⁻¹ uniconazole (S3307) and 40 mg L⁻¹ 5-aminolevulinic acid (ALA) were sprayed at the stage of nine expanded leaves. Our results showed that both S3307 and ALA applications significantly increased all ear width, volume, and mass in the maturity stage. Both applications also upregulated photosynthetic efficiency *via* increasing the chlorophyll content, net photosynthetic rate, transpiration rate, and stomatal conductance, as well as reduced the intercellular CO₂ concentration after the silking stage. In addition, both applications upregulated further the antioxidant system *via* enhancing the activity of antioxidants and contents of soluble protein and sugar, as well as reducing the malondialdehyde content after the silking stage. Thus, both S3307 and ALA applications can improve maize yield in saline-alkali land *via* enhancing ear morphology and increasing photosynthetic efficiency and antioxidants.

Keywords: leaf senescence; photosynthesis; plant growth regulator; *Zea mays* L.

Introduction

Crops are subjected to various abiotic and biological stresses during their growth process. Saline-alkali stress, being one of the serious abiotic stresses, is caused by

land salinization with an increasing tendency, leading to the poor growth and production loss of crops (Menezes-Benavente *et al.* 2004, Wani and Sah 2014, Saghafi *et al.* 2018, 2019). At present, the total area of saline-alkali land in the world is about 960 million hectares, accounting

Highlights

- S3307 and ALA applications upregulated photosynthetic capacity of maize
- S3307 and ALA applications increased maize yield by improving ear morphology
- S3307 and ALA applications upregulated antioxidants activity of maize in saline-alkali land

Received 28 January 2022

Accepted 13 June 2022

Published online 12 July 2022

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Abbreviations: ABA – abscisic acid; ANOVA – analysis of variance; APX – ascorbate peroxidase; CAT – catalase; Chl – chlorophyll; C_i – intercellular CO₂ concentration; E – transpiration rate; EC – electrical conductivity; GA – gibberellins; GPS – global position system; g_s – stomatal conductance; MDA – malondialdehyde; NBT – nitroblue tetrazolium chloride; OM – organic matter; PGRs – plant growth regulators; P_N – net photosynthetic rate; POD – peroxidase; ROS – reactive oxygen species; SOD – superoxide dismutase; TBARS – thiobarbituric acid reactive substances.

Acknowledgments: The authors are grateful for the support of Heilongjiang Bayi Agricultural University and the Heilongjiang Academy of Agricultural Sciences. This research was supported by the Youth Program of the Heilongjiang Academy of Agricultural Sciences (2021YYYYF047).

Conflict of interest: The authors declare that they have no conflict of interest.

for 9.4% of the global land area (Nishiuchi *et al.* 2007). China is one of the countries with the most serious soil salinization, which is characterized by the traits of wide distribution, high degree, complexity and diversity, and so on. These saline-alkali lands are mainly distributed in the northeast, north, inland regions of northwest, and coastal plain areas in China, with a total area of about 100 million hectares (Liu and Wang 2021).

Leaf photosynthesis is generally considered the main source that accumulates organic matter in crops, and its contribution rate to grain yield is more than 90% after the silking stage (Wang *et al.* 2016). Therefore, this period plays a vital role in yield production (Yamori *et al.* 2010). Meanwhile, leaves gradually become senescent after the silking stage. Indeed, leaf senescence is a widespread natural phenomenon, however, the saline-alkali stress can severely influence the speed and time of leaf senescence (Allu *et al.* 2014). Saline-alkali stress destroys the ion balance of plants, resulting in osmotic stress and water deficiency, which ultimately reduces photosynthetic rate and yield (Alam *et al.* 2019). In addition, saline-alkali stress induces the production of reactive oxygen species (ROS), leads to the disintegration of membrane systems, and inhibits plant growth (Saghafi *et al.* 2018, Feng *et al.* 2021). To overcome the physiological stress by the saline-alkali environment, plants activate antioxidant defense systems which include superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), and other enzyme antioxidants to neutralize and counteract the deleterious effects of ROS (Liu *et al.* 2013, Wang *et al.* 2018, Zhao *et al.* 2019). Leaf senescence is accompanied by decreasing chlorophyll (Chl) content, soluble sugar, and soluble protein, and increasing the MDA accumulation. Leaf senescence affects PSII of maize and is mainly manifested in the obstruction of stomatal opening and closure, leading to a decreased net photosynthetic rate, transpiration rate, and stomatal conductance, but increases intercellular CO₂ concentration (Ahmad *et al.* 2021a). Due to the decrease in photosynthesis, maize cannot accumulate and transform organic matter normally, thereby decreasing the maize yield (Ren *et al.* 2016, Kamran *et al.* 2020). Therefore, the strategies of improving the activities of antioxidants and alleviating the oxidative damage are essential for prolonging leaf life span and promoting plant adaptability, to increase maize yield in saline-alkali land (Menezes-Benavente *et al.* 2004).

Plant growth regulators (PGRs) are generally considered a tool to improve crop yield by enhancing its photosynthetic capacity and delaying its senescence and stresses (Chen *et al.* 2021). Uniconazole (S3307), a high-efficiency PGR, can inhibit gibberellin (GA) and abscisic acid (ABA) biosynthesis (Saito *et al.* 2006). Meanwhile, S3307 protects crops from various stresses, including salinity (Al-Rumaih 2007), drought (Ahmad *et al.* 2019a, Jiang *et al.* 2020), waterlogging (Qiu *et al.* 2005), low temperature (Zhao *et al.* 2019), and high temperature (Fletcher and Hofstra 1990). In addition, it regulates the content of endogenous hormones, increases photosynthetic pigments, photosynthetic efficiency, antioxidant capacity, and starch accumulation (Liu *et al.*

2019), improving crop quality and yield (Ahmad *et al.* 2018). 5-aminolevulinic acid (ALA) is a new type of PGR with nontoxic side effects and easy degradation, which is the precursor of all porphyrin biosynthesis and the key precursor of plant Chl synthesis (Sasikala *et al.* 1994, Reinbothe and Reinbothe 1996). Since it can regulate plant growth and development, ALA has been widely applied in numerous crops, including rape (Liu *et al.* 2013, Ali *et al.* 2015), wheat (Ostrowska *et al.* 2019), tomato (Zhao *et al.* 2015), maize (Kaya and Ashraf 2021), and rice (Li *et al.* 2011). Also, ALA improves crops' photosynthetic efficiency and protects crops from various stresses. Liu *et al.* (2013) reported that ALA enhances photosynthetic gas exchange, Chl fluorescence, and antioxidant system in oilseed rape. Kaya and Ashraf (2021) showed that ALA supplementation increased maize salt tolerance, which in turn played a promising role in upregulating Chl content and ROS activity. Moreover, Naeem *et al.* (2011) showed that ALA ameliorates salinity stress in *Brassica napus* L. by improving soluble protein, soluble sugar, and ROS, leading to an increase in leaf area. Previous studies suggest that S3307 and ALA have been widely used to increase photosynthetic efficiency, as well as against abiotic stress, improving crop yield. However, there are few reports of S3307 and ALA regulating maize yield in saline-alkali areas, especially on the photosynthetic efficiency change under saline-alkali stress and senescence after the silking stage. Thus, the present investigation was conducted to determine whether S3307 and ALA increase the yield of maize planted in saline-alkali land and whether such increase is correlated with changes in photosynthetic efficiency, antioxidant capacity, and ear morphology. The experimental results will provide a theoretical basis for the chemical regulation of maize cultivated in saline-alkali areas.

Materials and methods

Study site and materials: A series of field experiments were performed at the Daqing Branch of Heilongjiang Academy of Agricultural Sciences in Northeast China (46°40'N, 125°14'E) in 2019 and 2020. It was a typical saline-alkali soil. The physicochemical properties of topsoils (0–20 cm) were as follows:

Soil characteristics	Value
pH	8.3
OM [g kg ⁻¹]	26.2
Total N [g kg ⁻¹]	2.9
Available P [mg kg ⁻¹]	17.7
Available K [mg kg ⁻¹]	119.8
EC [μS cm ⁻¹]	102.9
Bulk density [g cm ⁻³]	1.2
Porosity [%]	54.8
Water content [%]	14.1

The soil sampling plot was equally spaced into 40 × 40-m grids, then at five sampling points, the soil was

mixed as the representing sample with a sampling depth of (0–20 cm) in one grid. The geographical location information of the sampling point was accurately recorded by a global position system (GPS) locator. The soil pH was determined by a pH meter (*FE20 Plus*, METTLER TOLEDO Corporation, Shanghai, China) with soil:water = 1:2.5. The $K_2Cr_2O_7-H_2SO_4$ external heating method was used to test the organic matter (OM) content (Lu 1999). The semi-trace Kjeldahl method was adopted to analyze the total nitrogen (N) content (Zhang *et al.* 2011). The content of available phosphorus (P) was determined by using the method mentioned by Zhang *et al.* (2011). We tested the available potassium (K) by using the flame photometer method (Lu 1999). Bulk density, porosity, and water content were tested using the method mentioned by Bao (2005). Maize was planted in early May and harvested at the end of September. The rainfall and temperature during the two growing seasons are presented in Fig. 1.

Xianyu 335 (XY335) and Nendan 18 (ND18), two main local maize varieties, were used as experimental materials, which were provided by the Daqing Branch of Heilongjiang Academy of Agricultural Sciences. The plant growth regulators tested were uniconazole and 5-aminolevulinic acid, which were provided by the Chemical Control Laboratory of Heilongjiang Bayi Agricultural University.

Experiment design: According to the screening tests of the applicable concentration of PGRs in our preliminary experiment, the optimum spraying concentration was 25 mg(S3307) L^{-1} (Ahmad *et al.* 2021a) and 40 mg(ALA) L^{-1} (Beyzaei *et al.* 2015), with tap water as the control (CK). Foliar spraying at the stage of nine expanded leaves (225 $L ha^{-1}$) was carried out at about 17:00 h in the sunny and windless afternoon. The planting density of XY335 and ND18 was 60,000 plants ha^{-1} and 75,000 plants ha^{-1} , respectively. The experimental plot had eight rows, the length was 12 m, the row spacing was 0.65 m, the interval aisle was 1 m, and the area of the plot was 62 m^2 . The experiment was repeated three times in a completely randomized block design, with a total of six treatments (XY335 + S3307, XY335 + ALA, XY335 +

CK, ND18 + S3307, ND18 + ALA, ND18 + CK). Field fertilization, weeding, and management were the same as local conventional production.

Measurements: In the experiment, maize ear leaves were sampled and measured at six stages which include VT (tasseling), R1 (silking stage), R2 (blister stage, 7 d after the silking stage), R3 (milk stage, 14 d after the silking stage), R4 (21 d after the silking stage), and R5 (physiological maturity stage). In one pilot area, five ear leaves were cut and stored in $-80^{\circ}C$ liquid nitrogen for the determination of Chl, MDA, soluble protein, soluble sugar, and antioxidant enzymes.

Chl content: The Chl contents including Chl *a*, Chl *b*, and Chl (*a+b*) were determined based on the method described by Arnon (1949) but slightly improved. Fresh leaves (0.1 g) were cut and put into 10 mL of anhydrous ethanol until the leaves fade to colorless under dark conditions. The optical density of the solution was measured spectrophotometrically (*UV-3600 Plus*, Shimadzu, Japan) at 663 and 645 nm and Chl contents were calculated as follows: Chl *a* = $12.72 A_{663} - 2.59 A_{645}$, Chl *b* = $22.88 A_{645} - 4.67 A_{663}$, Chl (*a+b*) = $20.29 A_{645} + 8.05 A_{663}$, where *A* represents the absorption at the referenced wavelength (663 and 645 nm).

Gas-exchange parameters: Net photosynthetic rate (P_N), transpiration rate (E), intercellular CO_2 concentration (C_i), and stomatal conductance (g_s) were detected using an *LI-6400 XT* portable photosynthesis system (*Li-Cor Inc.*, Nebraska, USA) under irradiance of 1,000 $\mu mol(photon) m^{-2} s^{-1}$ and CO_2 concentration of 500 $\mu mol s^{-1}$. All treatments were performed from 9:00 to 11:00 h on a clear and sunny day.

Antioxidant enzyme activity: Approximately 0.50 g of maize leaves was placed in liquid nitrogen and then ground; 10 mL (0.05 mol L^{-1}) of pre-cooled phosphate buffer (pH 7.8) was added and then the mixture was centrifuged at $12,000 \times g$ for 15 min at $4^{\circ}C$. The supernatant was used to determine enzyme activity and malondialdehyde (MDA) content.

Briefly, the superoxide dismutase (SOD, EC 1.15.1.1) and peroxidase (POD, EC 1.11.1.7) activities of maize leaves were determined using the nitroblue tetrazolium (NBT) and oxidized guaiacol by following the procedure developed by Giannopolitis and Ries (1977) and Choudhary (2011), respectively. The SOD activity unit (U) was defined as the amount of enzyme required to inhibit 50% NBT reduction as monitored at 560 nm. The SOD activity was expressed as U $g^{-1}(FM)$. One unit (U) of the POD activity was defined as optical density (OD) value increases 0.01 per min as monitored at 470 nm. The POD activity was expressed as U $mg^{-1}(FM) min^{-1}$. In addition, catalase (CAT, EC 1.11.1.6) activity assessment was obtained according to the method of Fu and Huang (2001), and ascorbate peroxidase (APX, EC 1.11.1.11) activity was measured using the method of Cakmak and Marschner (1992). One unit (U) of the CAT and APX activity was defined as OD value decreases 0.01

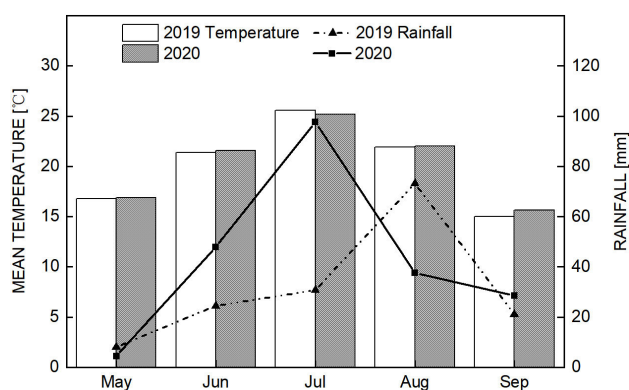


Fig. 1. Mean temperature [$^{\circ}C$] and rainfall [mm] during the 2019 and 2020 growing seasons.

per minute as monitored at 240 and 290 nm, respectively. The CAT and APX activities were both expressed as $U\ mg^{-1}(FM)\ min^{-1}$.

MDA, soluble protein, and soluble sugar: According to the method of Zahir *et al.* (2012), the MDA concentrations of maize leaves were quantified by the thiobarbituric acid reaction. Soluble sugars were evaluated using the anthrone method of Hu *et al.* (2009). Soluble protein was assayed by the method of Bradford (1976).

Ear morphology and yield components: At the maturity stage, 25 continuous ears of maize were randomly selected in each plot, and ten representative ears of maize were selected to measure the ear morphology indexes (*i.e.*, ear length and width, bald length, ear volume, ear fresh mass, ear dry mass) and yield components (*i.e.*, row number per ear, grain number per row, hundred-grain mass). The grain yield of maize was represented at 14.0% moisture content.

Statistical analysis: All data were produced using *Microsoft Excel* (2010) and all figures were processed by *Origin 12.0* (*OriginLab Corporation*, USA). Moreover, all experiment data were performed with analysis of variance (ANOVA) and Duncan's multiple comparison test using *Statistical Product and Service Solutions (SPSS 20.0, IBM Corporation, USA)* to determine significant differences between different treatments ($p < 0.05$).

Results

Grain yield and its components: Two-year experimental results showed that the PGRs treatment markedly increased the grain yield (Table 1). With both S3307 and ALA application, the grain yield of XY335 increased by 8.1 and 12.0% in 2019, and 11.9 and 13.9% in 2020 compared to control, respectively. With these two applications, the grain yield of ND18 increased by 7.6 and 9.0% in 2019,

and 15.5 and 16.4% in 2020, respectively. Meanwhile, the regulators' treatments markedly increased maize yield components (*i.e.*, kernel number per ear and hundred-grain mass). With S3307 and ALA application, kernel number per ear of XY335 increased by 5.9 and 8.5% in 2019, and 7.6 and 9.0% in 2020 compared to control, respectively. The kernel number per ear of ND18 increased by 5.7 and 6.4% in 2019 and 9.7 and 8.5% in 2020, respectively. With S3307 and ALA application, the hundred-grain mass of XY335 increased by 2.0 and 3.3% in 2019, and 3.9 and 4.0% in 2020 compared with control, respectively. With S3307 and ALA application, ND18 was increased by 1.7 and 2.5% in 2019, and 4.3 and 6.4% in 2020, respectively.

Ear size: Ear size being correlated with grain yield was affected markedly by PGRs (Table 2). The results showed that S3307 treatment markedly increased ear width and volume, but reduced bald length, while ALA treatment markedly increased ear length and ear volume. First, the ear lengths of both XY335 and ND18 under the ALA treatment were markedly higher than those under the S3307 treatment and increased by 8.8 and 9.0% compared to CK, respectively. Second, the ear width of both XY335 and ND18 under the S3307 treatment markedly increased by 9.2 and 10.3% compared to CK, respectively. Then, the bald lengths of both XY335 and ND18 under the S3307 treatment decreased by 35 and 30%, respectively, all of which were markedly lower than those under the ALA treatment. Last, the ear volumes of both XY335 and ND18 under the S3307 and ALA treatment markedly increased by 6.5 and 8.6% and 9.5 and 12.1% compared to CK, respectively.

Ear mass: Ear mass correlated with grain yield was markedly affected by PGRs (Table 2). Both S3307 and ALA treatments markedly increased the ear fresh mass and dry mass of both varieties. The ear fresh mass of XY335 and ND18 under both S3307 and ALA treatments

Table 1. Effects of S3307 and ALA on maize grain yield and yield components during 2019 and 2020. S3307 – foliar uniconazole application; ALA – foliar 5-aminolevulinic acid application. Values are the means \pm SE of three repetitions ($n = 3$). Different lowercase letters indicate significant differences at $P < 0.05$ (LSD test).

Year	Varieties	Treatment	Row number per ear	Grain number per row	Kernel number per ear	100-grain mass [g]	Yield [kg ha ⁻¹]
2019	XY335	S3307	16.60 \pm 0.23 ^a	37.63 \pm 0.19 ^b	624.63 \pm 5.93 ^a	33.97 \pm 0.12 ^a	11.55 \pm 0.15 ^a
		ALA	16.50 \pm 0.15 ^a	38.73 \pm 0.24 ^a	639.03 \pm 2.88 ^a	34.40 \pm 0.06 ^a	11.96 \pm 0.03 ^a
		Control	16.20 \pm 0.35 ^a	36.37 \pm 0.18 ^c	589.10 \pm 11.92 ^b	33.30 \pm 0.20 ^b	10.68 \pm 0.25 ^b
	ND18	S3307	14.70 \pm 0.06 ^a	37.53 \pm 0.09 ^b	551.73 \pm 1.64 ^a	36.17 \pm 0.12 ^a	12.88 \pm 0.08 ^a
		ALA	14.47 \pm 0.07 ^a	38.37 \pm 0.19 ^a	555.01 \pm 0.43 ^a	36.43 \pm 0.13 ^a	13.05 \pm 0.06 ^a
		Control	14.17 \pm 0.09 ^b	36.83 \pm 0.27 ^c	521.85 \pm 7.08 ^b	35.55 \pm 0.09 ^b	11.97 \pm 0.19 ^b
2020	XY335	S3307	16.64 \pm 0.18 ^a	39.70 \pm 0.30 ^a	660.71 \pm 7.67 ^a	37.11 \pm 0.21 ^a	12.72 \pm 0.19 ^a
		ALA	16.35 \pm 0.18 ^a	40.92 \pm 0.51 ^a	669.26 \pm 15.24 ^a	37.15 \pm 0.48 ^a	12.95 \pm 0.23 ^a
		Control	16.17 \pm 0.17 ^a	38.00 \pm 0.58 ^b	614.17 \pm 4.97 ^b	35.72 \pm 0.11 ^b	11.37 \pm 0.03 ^b
	ND18	S3307	15.76 \pm 0.24 ^a	37.95 \pm 0.32 ^{ab}	597.82 \pm 4.79 ^a	35.74 \pm 0.28 ^a	13.62 \pm 0.18 ^a
		ALA	15.17 \pm 0.44 ^a	39.00 \pm 0.58 ^a	591.17 \pm 13.04 ^a	36.44 \pm 0.30 ^a	13.72 \pm 0.26 ^a
		Control	14.67 \pm 0.17 ^a	37.17 \pm 0.17 ^b	545.08 \pm 5.38 ^b	34.26 \pm 0.15 ^b	11.79 \pm 0.08 ^b

increased by 5.4 and 8.1%, and 6.2 and 8.6% compared to CK, respectively. Also, the ear dry mass of XY335 and ND18 under S3307 and ALA treatment significantly increased by 9.1 and 10.3% and 9.2 and 16.7% compared to CK, respectively.

Chl content: Photosynthetic capacity has an important effect on yield, while Chl, being the main pigment of

photosynthesis, affects photosynthetic capacity. After the silking stage, the content of Chl declined gradually accompanied by leaf aging. We found that applying both S3307 and ALA significantly improved the Chl content (Fig. 2). Our experimental results showed that although the applied regulators could not completely prevent Chl degradation in maize leaves, they effectively alleviated the degradation trend during the leaf senescence process.

Table 2. Effects of S3307 and ALA on maize ear morphology. S3307 – foliar uniconazole application; ALA – foliar 5-aminolevulinic acid application. Values are the means \pm SE of three repetitions ($n = 3$). Different lowercase letters indicate significant differences at $P < 0.05$ (LSD test).

Varieties	Treatment	Ear length [cm]	Ear width [cm]	Bald length [cm]	Ear volume [mL]	Ear fresh mass [g]	Ear dry mass [g]
XY335	S3307	21.38 \pm 0.31 ^b	4.77 \pm 0.03 ^a	1.43 \pm 0.13 ^b	382.00 \pm 1.53 ^a	405.67 \pm 5.36 ^{ab}	327.33 \pm 8.97 ^a
	ALA	22.49 \pm 0.29 ^a	4.62 \pm 0.19 ^{ab}	1.77 \pm 0.15 ^a	389.44 \pm 2.70 ^a	416.33 \pm 8.69 ^a	331.00 \pm 6.66 ^a
	Control	20.67 \pm 0.33 ^b	4.37 \pm 0.07 ^b	2.20 \pm 0.17 ^a	358.67 \pm 7.31 ^b	385.00 \pm 7.57 ^b	300.00 \pm 10.00 ^a
ND18	S3307	17.83 \pm 0.44 ^b	4.60 \pm 0.06 ^a	1.47 \pm 0.07 ^b	320.66 \pm 1.45 ^b	348.33 \pm 2.73 ^{ab}	276.67 \pm 3.33 ^{ab}
	ALA	19.26 \pm 0.26 ^a	4.33 \pm 0.17 ^{ab}	2.00 \pm 0.15 ^a	328.33 \pm 0.96 ^a	356.33 \pm 5.89 ^a	295.67 \pm 8.69 ^a
	Control	17.67 \pm 0.33 ^b	4.17 \pm 0.09 ^b	2.10 \pm 0.20 ^a	292.78 \pm 2.42 ^c	328.00 \pm 8.02 ^b	253.33 \pm 8.82 ^b

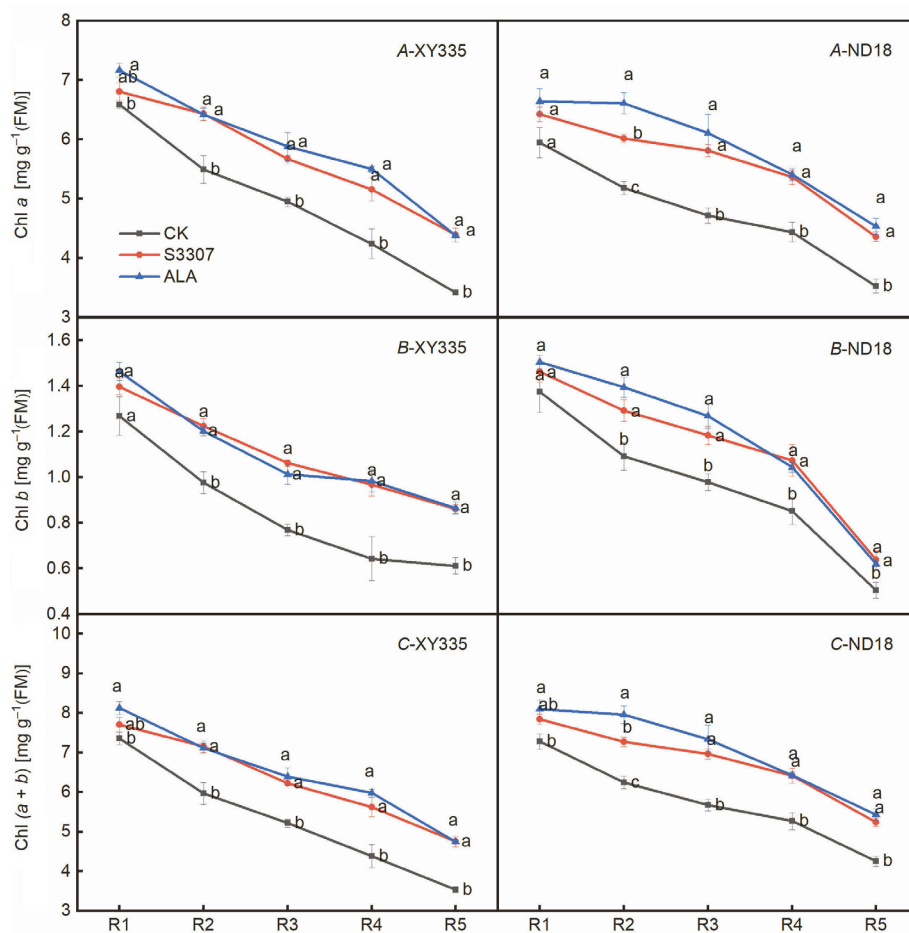


Fig. 2. Effects of S3307 and ALA on chlorophyll content in Xianyu-335 (XY335) and Nendan-18 (ND18) leaves at R1, R2, R3, R4, and R5 stages. (A) Chlorophyll *a* content (Chl *a*), (B) chlorophyll *b* content (Chl *b*), (C) chlorophyll (*a*+*b*) content [Chl (*a*+*b*)]. CK – control (tap water); S3307 – foliar uniconazole application; ALA – foliar 5-aminolevulinic acid application. Data represent means \pm SE. Different lowercase letters indicate significant differences at $P < 0.05$ (LSD test).

The contents of Chl *a*, Chl *b*, and Chl (*a*+*b*) of both XY335 and ND18 were the highest in the R1 stage, and there was no significant difference between regulators' treatments and control. However, the contents of Chl *a*, Chl *b*, and Chl (*a*+*b*) decreased gradually from the stages of R2 to R5 and showed a significant difference between the regulators' treatments and control. XY335 under both S3307 and ALA treatments significantly increased Chl *a* content (by 16.1–33.2%, 18.5–33.6%, respectively), Chl *b* content (by 25.4–50.3%, 23.1%–53.1%, respectively), and Chl (*a*+*b*) content (by 16.4–23.0%, 19.3–36.5%, respectively) (Fig. 2A–C, left). ND18 under both S3307

and ALA treatments markedly increased Chl *a* content (by 16.1–23.6%, 21.9–29.4%, respectively), Chl *b* content (by 18.1–25.6%, 22.0–29.2%, respectively) and Chl (*a*+*b*) content (by 16.4–23.0%, 21.9–29.4%, respectively) (Fig. 2A–C, right). The regulation effect under ALA treatment was superior to S3307, but there was no significant difference between the two PGRs.

Gas-exchange parameters: After the silking stage, P_N , g_s , and E gradually decreased, but C_i gradually increased, all of which illustrated the effects of aging on gas exchange and CO₂ absorption. In the present study, the applications

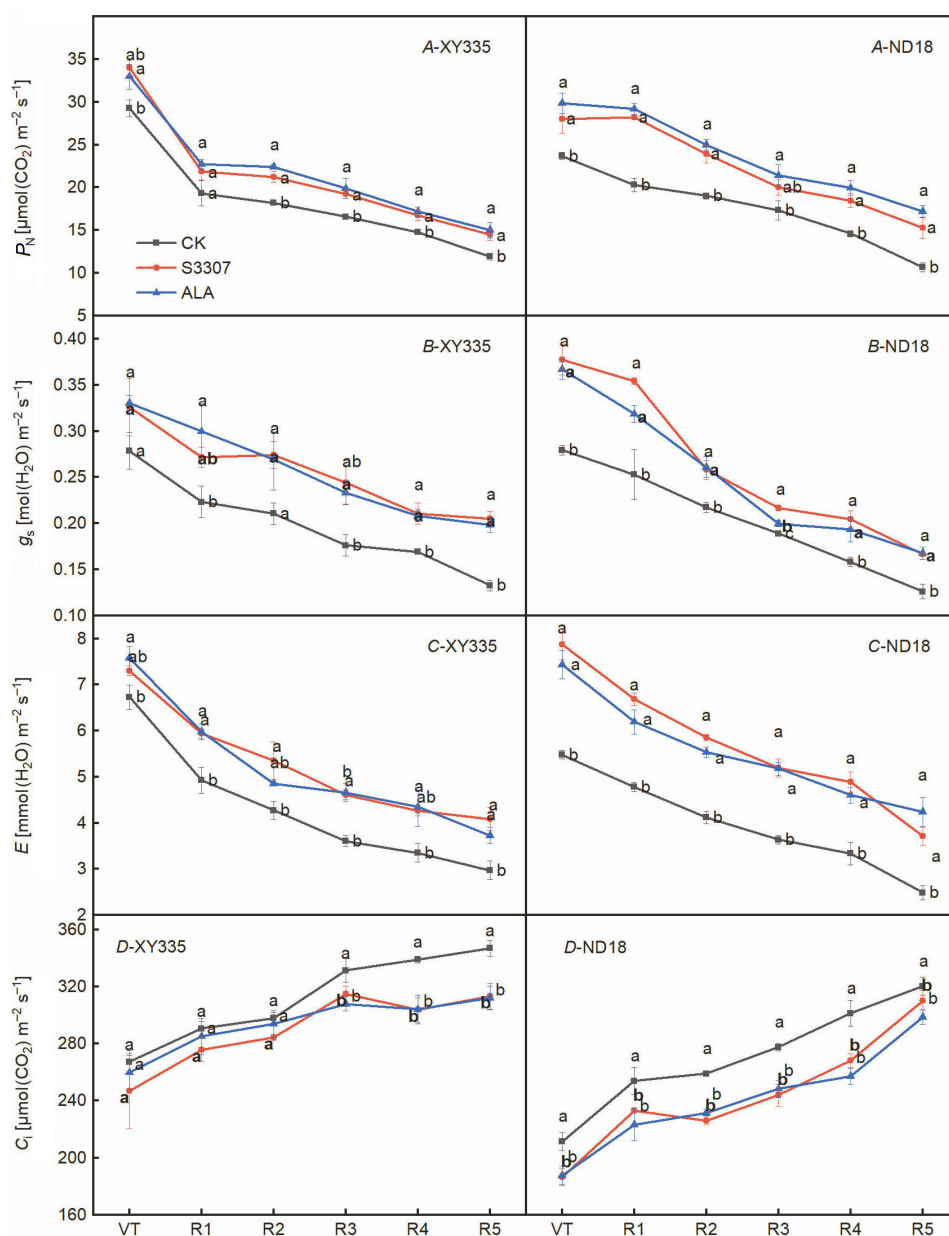


Fig. 3. Effects of S3307 and ALA on gas exchange parameters in Xianyu-335 (XY335) and Nendan-18 (ND18) leaves at stages of VT, R1, R2, R3, R4, and R5. (A) Net photosynthetic rate (P_N), (B) stomatal conductance (g_s), (C) transpiration rate (E), (D) intercellular CO₂ concentration (C_i). CK – control (tap water); S3307 – foliar uniconazole application; ALA – foliar 5-aminolevulinic acid application. Data represent means \pm SE. Different lowercase letters indicate significant differences at $P < 0.05$ (LSD test).

of PGRs on XY335 and ND18 markedly increased P_N , g_s , and E , but decreased C_i (Fig. 3). The applications of S3307 and ALA markedly increased P_N , which increased from 13.2 to 21.4%, from 16.3 to 25.8% during the R2–R5 stages in XY335, respectively, and from 15.4 to 43.2%, and from 23.5 to 61.0% during all reproductive development stages of ND18, respectively, relatively to CK (Fig. 3A). ALA treatment on this effect was better than that of S3307 treatment, but there was no significant difference between the two regulators. The application of S3307 and ALA markedly increased g_s at R4 (by 24.4 and 23.2%, respectively) and R5 (by 54.6 and 49.4%, respectively) stages of XY335, and all reproductive development stages (by 14.7–40.0%, and 5.7–33.2%, respectively) of ND18, compared to CK (Fig. 3B). The application of both S3307 and ALA markedly increased E at R2 (by 25.3 and 13.7%, respectively) and R5 (by 37.4 and 25.6%, respectively) stages of XY335 and all reproductive development stages (by 39.9–49.6%, 29.6–70.6%) of ND18, compared to CK (Fig. 3C). In contrast, the application of both S3307 and ALA markedly decreased C_i at R3–R5 (by 5.0–10.4%, and 7.0–10.3%, respectively) stages of XY335 and VT–R4 (by 8.1–12.7%, and 6.7–14.6%, respectively) stages of ND18, compared to CK (Fig. 3D).

Antioxidant enzyme activity: The activity of antioxidant enzyme systems represents the anti-aging ability of plants, as it can effectively delay the senescence of plant leaves. In this study, the applications of S3307 and ALA markedly increased the activities of antioxidant enzymes in maize leaves. The activity of SOD in all treatments increased gradually from R1 to R2 stages and then decreased from R2 to R5 stages. XY335 under both S3307 and ALA applications markedly increased SOD activity at R2–R5 stages, compared to control (Fig. 4A, left). ND18 under both the S3307 and ALA applications markedly increased the SOD enzyme activity at all stages (by 27.2–55.3% and 28.0–44.7%, respectively) (Fig. 4A, right).

POD is another important antioxidant enzyme. In this study, the POD activities increased markedly after foliar spraying of both S3307 and ALA. After the application of S3307 and ALA, the POD activity of XY335 gradually increased from R1 to R3 stages, and then decreased from R3 to R5 stages. The highest increase in POD activity under the application of S3307 and ALA was found at R3 (by 51.8 and 51.9%, respectively), R4 (by 53.8 and 51.6%, respectively), and R5 (by 71.2 and 75.0%, respectively) stages, compared with control (Fig. 4B). After the application of both S3307 and ALA, the POD activity of ND18 gradually increased from R1 to R2 stages, and then decreased from R2 to R5 stages. The POD activity under the application of S3307 and ALA markedly increased at R1–R5 stages compared with control (Fig. 4B).

Based on the results of Fig. 4, the activity of CAT increased markedly after foliar spraying of both S3307 and ALA. The activity of CAT in all treatments increased gradually from R1 to R3 stages and then decreased from R3 to R5 stages. In comparison with control, XY335 under the application of both S3307 and ALA increased the CAT activity at R1–R4 stages (by 25.0–57.5% and 30.0–56.1%,

respectively) (Fig. 4C). ND18 under the application of both S3307 and ALA increased the CAT activity at R2–R5 stages (by 32.5–74.3% and 42.5–68.6%, respectively) (Fig. 4C).

The activity of APX increased significantly after foliar spraying of both S3307 and ALA. After the application of both S3307 and ALA, APX activity of XY 335 gradually increased from R1 to R3 stages and then decreased from R3 to R5 stages; the APX activity increased at all stages (by 23.3–45.2% and 17.4–47.6%, respectively), compared with control (Fig. 4D). After the application of S3307 and ALA, APX activities of ND18 gradually increased from R1 to R2 stages and then decreased from R2 to R5 stages, which increased the APX activity at all stages (R1–R5), compared with control (Fig. 4D).

MDA, soluble protein, and soluble sugar: In this present study, maize leaf senescence after the silking stage showed a gradual increase in MDA content, but the regulator markedly decreased its content as compared to the control. XY335 under the application of S3307 and ALA markedly decreased MDA at all stages (by 15.6–21.2% and 8.8–19.6%, respectively). ND18 under the application of both S3307 and ALA markedly decreased MDA content at all stages (by 20.4–27.4% and 17.0–26.1%, respectively) (Fig. 5).

Although the soluble protein content of maize leaves decreased markedly with the process of leaf senescence, it markedly increased with the application of regulators to the leaves after the silking stage (Fig. 6). XY335 under the application of S3307 and ALA increased the soluble protein content at all stages (by 23.8–72.7% and 24.2–59.5%, respectively) (Fig. 6A, left). ND18 under the application of both S3307 and ALA increased the soluble protein content at all stages (by 14.8–27.4% and 20.7–51.6%, respectively) (Fig. 6A, right).

The soluble sugar content gradually increased from R1 to R2 stages and then decreased from R2 to R5 stages, but it was markedly increased with the application of regulators to the leaves after the silking stage (Fig. 6). XY335 under the application of both S3307 and ALA markedly increased soluble sugar content at R1 and R2 stages (Fig. 6B, left). Also ND18 under the application of S3307 and ALA markedly increased soluble sugar content at R1 and R2 stages (Fig. 6B, right).

Discussion

At the silking stage, maize leaves begin aging, which is triggered by the interaction between the external environment and internal metabolism (Wang *et al.* 2016). However, saline-alkali stress promotes leaf aging (Allu *et al.* 2014). Accompanying the loss of photosynthetic pigment, the leaf senescence decreases photosynthetic efficiency and yield (Yan *et al.* 2015). Since it plays an important role in the absorption and transmission of light energy in natural conditions, Chl is considered one of the most important pigments in photosynthesis (Ali *et al.* 2013). However, its content gradually decreases during leaf senescence (Kamran *et al.* 2020, Wu *et al.*

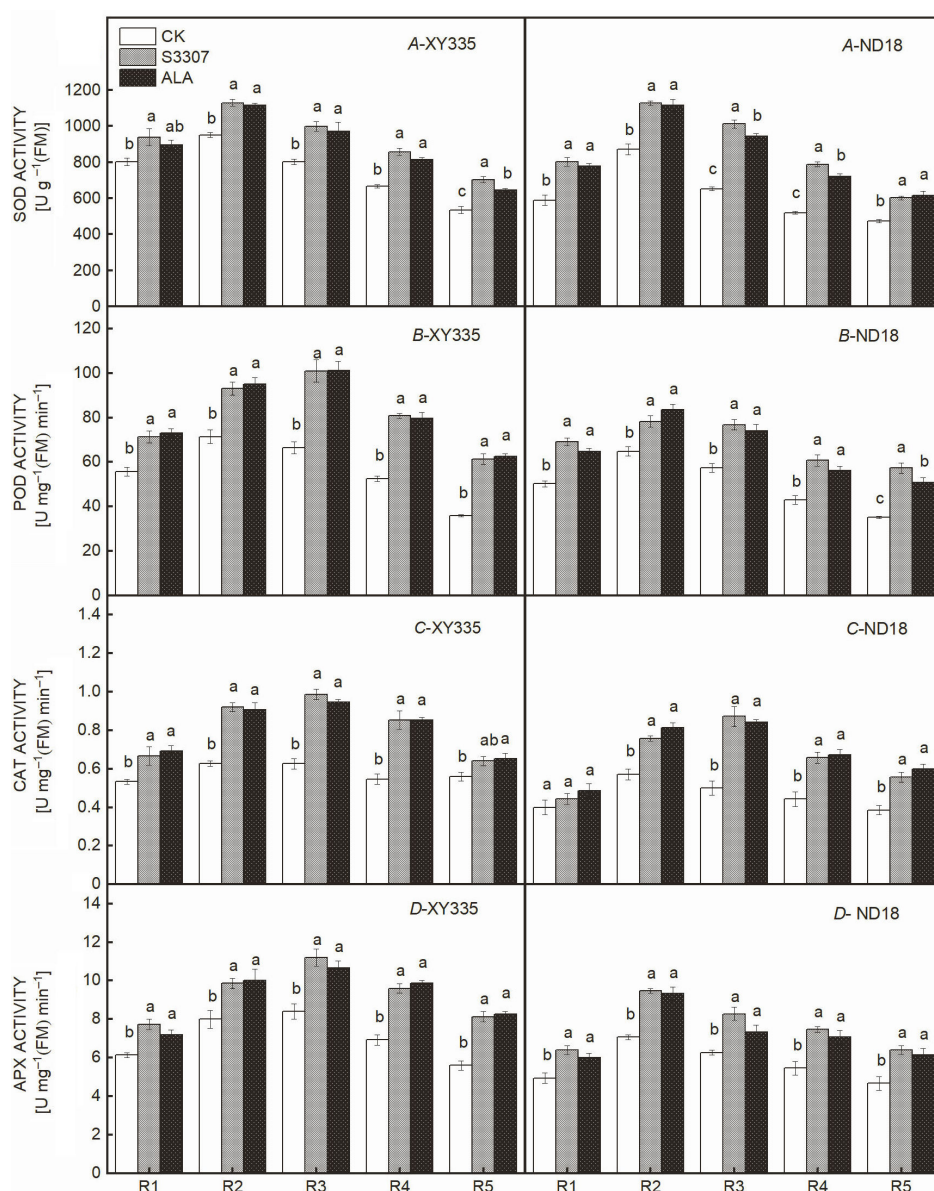


Fig. 4. Effects of S3307 and ALA on the activities of the antioxidant enzymes in Xianyu-335 (XY335) and Nendan-18 (ND18) leaves at R1, R2, R3, R4, and R5 stages. (A) Superoxide dismutase activity (SOD), (B) peroxidase activity (POD), (C) catalase activity (CAT), (D) ascorbate peroxidase activity (APX). CK – control (normal water); S3307 – foliar uniconazole application; ALA – foliar 5-aminolevulinic acid application. Data represent means \pm SE. Different lowercase letters indicate significant differences at $P < 0.05$ (LSD test).

2021). PGRs have a good regulatory effect on plant growth and development (Liu *et al.* 2013, Feng *et al.* 2021, Kai *et al.* 2021, Zhou *et al.* 2021). Our studies showed that applying S3307 and ALA increased the Chl content of maize leaves to promote the formation of yield *via* maintaining high photosynthetic activity and delaying leaf senescence. This finding was consistent with previous results. Yan *et al.* (2015) reported that uniconazole effectively suppressed excessive vegetative growth of soybean during the flowering stage, delayed senescence of photosynthetically active leaves at the pod-setting stage, and induced higher yield, which was involved with the

photosynthetic rate and Chl content changes. Ali *et al.* (2013) suggest that the application of ALA is beneficial to improving the rape leaf growth, Chl content, and photosynthetic gas-exchange capacity.

Adversities (*i.e.*, aging, low temperature, drought, salt stress, *etc.*) influence photosynthetic efficiency *via* driving gas exchange and the ability of crops to absorb and utilize CO_2 and thus reduce crop yield (Liu *et al.* 2013, Wang *et al.* 2017, Zhao *et al.* 2019, Ahmad *et al.* 2020, Feng *et al.* 2021). Previous studies showed that P_N in plant leaves decreased along with g_s and E , but C_i increased gradually under stress (Ahmad *et al.* 2021a, Wu *et al.* 2021). After

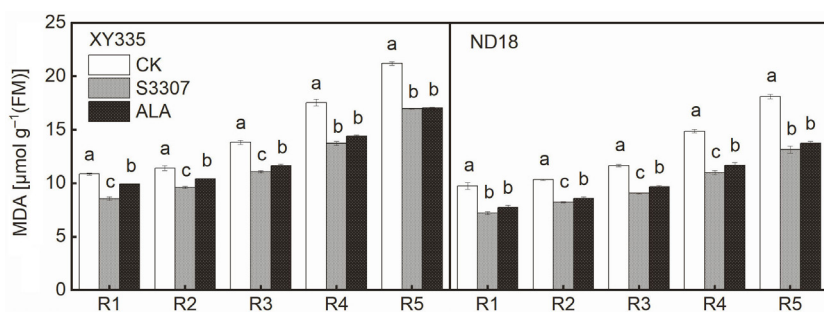


Fig. 5. Effects of S3307 and ALA on the malondialdehyde (MDA) content in Xianyu-335 (XY335) and Nendan-18 (ND18) leaves at R1, R2, R3, R4, and R5 stages. CK – control (normal water); S3307 – foliar uniconazole application; ALA – foliar 5-aminolevulinic acid application. Data represent means \pm SE. Different lowercase letters indicate significant differences at $P < 0.05$ (LSD test).

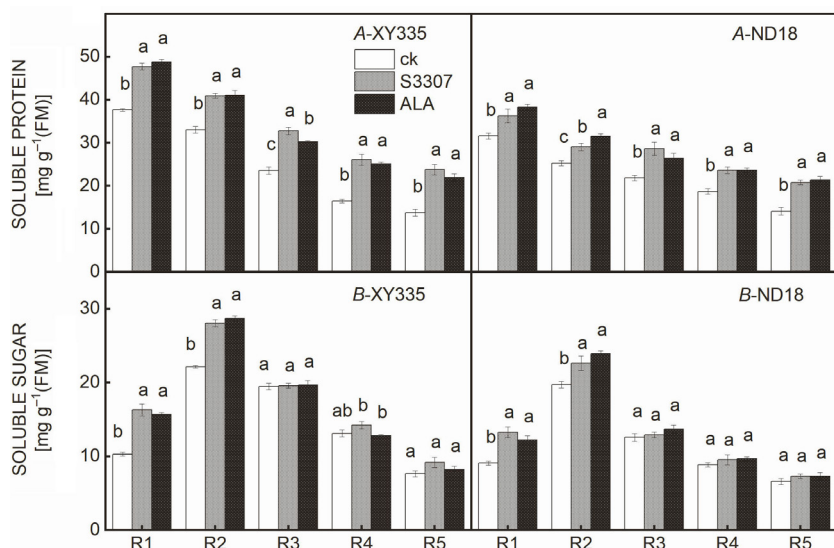


Fig. 6. Effects of S3307 and ALA on soluble protein and soluble sugar content in Xianyu-335 (XY335) and Nendan-18 (ND18) leaves at R1, R2, R3, R4, and R5 stages. (A) Soluble protein content (B), soluble sugar content. CK – control (normal water); S3307 – foliar uniconazole application; ALA – foliar 5-aminolevulinic acid application. Data represent means \pm SE. Different lowercase letters indicate significant differences at $P < 0.05$ (LSD test).

the silking stage, g_s and E gradually decreased. Due to leaf senescence, the cell was deficient in water, leading to the closure of stomata to prevent excessive water loss in the plant body. The senescence of photosynthetic apparatus in maize leaves leads to a decrease in CO_2 assimilation efficiency and an increase in intercellular CO_2 concentration (Ren *et al.* 2020). For example, the previous research reported that the S3307 and 6-benzylaminopurine (6-BA) could effectively improve the P_N , g_s , and E of soybean leaves, thus delaying the leaf senescence and increasing the yield (Liu *et al.* 2019). In this study, S3307 and ALA treatments also markedly slowed down the decline rate of P_N , g_s , and E and increased the C_i in maize leaves after silking stage, which plays a key role in maintaining stomatal opening, improving leaf carbon assimilation capacity and photosynthetic rate, helping to delay maize leaves senescence, therefore increasing the yield. The results are positive and in agreement with those studies of Ahmad *et al.* (2021a), which found the same phenomena. As illustrated in this study, compared with that of the two regulators, the regulation effect of ALA treatment was better than that of S3307, but there was no significant difference between the two regulators. Indeed, ALA treatment increased the Chl synthesis, absorption, and utilization capacity of CO_2 , the net photosynthetic rate. This reason may be attributed to ALA being the

precursor of Chl synthesis (Reinbothe and Reinbothe 1996). In addition, another possible reason is that ALA is a promoting regulator, which can increase the leaf area above the ear position, to improve photosynthetic efficiency and increase yield (Ali *et al.* 2013, Habiba *et al.* 2019).

Plant senescence is generally caused by the overproduction of ROS, while metabolic disorder of ROS is the process of plant senescence. With the metabolism, the plant produces harmful ROS in various ways, to destroy the structure and function of the cell membrane, resulting in cell death (Lam 2004). However, with its long-term evolution, a corresponding antioxidant protection system has been formed in organisms. This mechanism is to protect cells away from stress by reducing the accumulation of free radicals and scavenging excessive free radicals (Mittler *et al.* 2004). Indeed, SOD plays a central role in the plant defense system since it effectively converts reactive oxygen species into oxygen and hydrogen peroxide ($O_2^- + O_2^- + 2H^+ \rightarrow 2H_2O_2 + O_2$) (Mittler 2002). H_2O_2 is decomposed by CAT, POD, and APX to form molecular oxygen and water, which prevents membrane lipid peroxidation, delays plant senescence, and maintains normal growth and development. Ren *et al.* (2018, 2020) reported that the activities of SOD, POD, and CAT at the R3 to R5 stage of maize leaves after the silking stage decreased gradually in the waterlogged fields. Kamran

et al. (2020) also found that the activities of SOD, POD, CAT, and APX in maize leaves decreased gradually during 15 to 45 d after the silking stage. Similarly, our results illustrated that the activities of SOD, POD, CAT, and APX in maize leaves increased at first and then decreased in saline-alkali land. Indeed, PGRs application effectively increased the antioxidant enzyme activities and decreased membrane deterioration in leaves, which protected the functions of leaf chloroplasts and the integrity of cell membranes, thereby delaying leaf senescence. *Ahmad et al.* (2019b) reported that uniconazole seed soaking increased the activities of SOD, POD, and CAT in maize leaves, and delayed leaf senescence by improving the photosynthetic efficiency and yield of maize after the silking stage in semiarid regions. *Wang et al.* (2018) found that applying ALA increased the activities of antioxidant enzymes (SOD, POD, and CAT) in maize seedlings, thus increasing the resistance of maize to stress, photosynthetic efficiency, and organic matter accumulation. These findings were positively corresponding to previous reports that the application of S3307 and ALA makes maize possess a better ability to scavenge ROS for protecting biomolecules from oxidative damage, thus reducing the degradation of photosynthetic pigments and maintaining the photosynthetic function of plant leaves to increase yield.

MDA is the by-product of membrane lipid peroxidation, while its excessive accumulation in crops causes cell membrane damage, increasing cell permeability and loss of function (*Wang et al.* 2012). Therefore, the accumulation rate of MDA represents the ability of the tissue to scavenge free radicals. Indeed, the faster the accumulation of MDA in crop leaves, the slower the scavenging rate of free radicals, the greater the degree of cell membrane damage, and the faster the speed of plant senescence. For example, previous studies have demonstrated that the acceleration in the leaf senescence is always positively associated with the increase of MDA contents (*Wang et al.* 2016, *Kamran et al.* 2020). This phenomenon is similar to our results that found a continuous increase in endogenous contents of MDA with the advancement of leaf senescence after the silking stage in both XY335 and ND18. However, leaf MDA content in regulator-treated maize was lower than that in control. The application of both S3307 and ALA markedly decreased the MDA content after the silking stage in maize, indicating that regulator treatments alleviated the oxidative damage to the plants' membrane by decreasing the overproduction of MDA and enhancing the activity of antioxidants. These results are similar to the previous reports on both maize and soybean (*Ahmad et al.* 2019b, *Feng et al.* 2021, *Kaya and Ashraf* 2021).

Both soluble protein and sugar are not only important nutrients but also osmotic regulators while a decrease in contents of osmotic regulators is often accompanied by leaf senescence (*Peterson and Huffaker* 1975). In the present study, the application of both S3307 and ALA promoted the production of soluble protein and sugar in maize leaves to slow down their photosynthetic pigment degradation during senescence, indicating that regulator treatment improved the antioxidant capacity of plants.

Ahmad et al. (2021a) reported that the application of S3307 significantly increased soluble protein and delayed leaf senescence in maize at a high population density. *Wang et al.* (2018) suggest that exogenous application of ALA improved the soluble protein content of maize seedlings under low-temperature stress. In addition, both S3307 and ALA treatments markedly increased the soluble sugar content of XY335 and ND18 from the R1 to R2 stage, but there was no significant difference in R3 to R5 stage. The findings were in agreement with the results of previous studies, indicating that the regulator treatment effectively increases the content of soluble sugar and the antioxidant capacity in plants, but decreases soluble sugar after the R3 stage, which may be because of the increased transportation of soluble sugar from leaves to grains, impacting the increase of yield (*Wang et al.* 2016, *Kamran et al.* 2020).

Ear morphology was associated with grain yield but can be markedly affected by PGRs. Previous studies (*Ren et al.* 2018, *Liu et al.* 2021, *Wang et al.* 2016) pointed out that exogenous 6-BA, 2-diethylaminoethyl-3,4-dichlorophenylether (DCTPA), and diethyl aminoethyl hexanoate (DTA-6) improved maize yield by increasing ear length and diameter and reducing the bald length. A recent report also pointed out that uniconazole improved wheat yield by increasing ear mass (*Ahmad et al.* 2021b). In this study, both S3307 and ALA applications significantly increased all ear width, volume, and mass in the harvest. This is consistent with previous studies.

The yield components of maize mainly include ear number, grain number per ear, and grain mass. Of these factors, grain number per ear and grain mass are the decisive factors of yield (*Zhang et al.* 2017). In this study, the application of both S3307 and ALA markedly increased the grain yield of maize as compared to that of control. The higher yield was mainly related to the increase of hundred-grain mass and kernel number per ear, contributing to the increase of the dry matter accumulation rate through increasing Chl content, photosynthetic capacity, antioxidant enzymatic activity, and anti-aging ability in maize under the treatment of both S3307 and ALA. Similar findings indicated that maize yield was positively correlated with Chl content, photosynthetic rate, and antioxidant system, and negatively correlated with leaf senescence rate (*Ren et al.* 2016, *Wang et al.* 2016). A previous study has concluded that applying S3307 in semi-arid areas can effectively improve photosynthetic efficiency and delay leaf senescence to increase yield. This finding is in agreement with previous reports (*Ahmad et al.* 2018, 2021a). The application of both S3307 and 6-BA can effectively increase the Chl content, photosynthetic efficiency, and delay leaf senescence to increase the yield of maize, which was also consistent with a previous study (*Liu et al.* 2019).

Conclusion: Our experimental study indicated that uniconazole and 5-aminolevulinic acid could effectively improve photosynthetic efficiency and alleviate maize leaf senescence and saline-alkali stress after the silking stage to increase the maize yield. Comparative phenotypic and

physiological analysis revealed that the increase in maize yield may be attributed to the improvement of chlorophyll content, photosynthesis capacity, activation of antioxidant enzymes, and ear morphology. Our study thus, highlights that the uniconazole (25 mg L⁻¹) and 5-aminolevulinic acid (40 mg L⁻¹) application at the stage of nine expanded leaves can increase maize yield in saline-alkali land.

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