Exogenous application of growth stimulators improves the condition of maize exposed to soil drought

Agnieszka Ostrowska¹ · Maciej T. Grzesiak¹ · Tomasz Hura¹

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Abstract

Soil drought is a major problem in plant cultivation. This is particularly true for thermophilic plants, such as maize, which grow in areas often affected by precipitation shortage. The problem may be alleviated using plant growth and development stimulators. Therefore, the aim of the study was to analyze the effects of 5-aminolevulinic acid (5-ALA), zearalenone (ZEN), triacontanol (TRIA) and silicon (Si) on water management and photosynthetic activity of maize under soil drought. The experiments covered three developmental stages: three leaves, stem elongation and heading. The impact of these substances applied during drought stress depended on the plant development stage. 5-ALA affected chlorophyll levels, gas exchange and photochemical activity of PSII. Similar effects were observed for ZEN, which additionally induced stem elongation and limited dehydration. Beneficial effects of TRIA were visible at the stage of three leaves and involved leaf hydration and plant growth. A silicon preparation applied at the same developmental stage triggered similar effects and additionally induced changes in chlorophyll levels. All the stimulators significantly affected transpiration intensity at the heading stage.

Keywords Water stress · 5-aminolevulinic acid · Triacontanol · Silicon · Zearalenone

Abbreviations

ABS/CS _m	Amount of light energy absorption	P _N	Photosynthetic rate
5-ALA	5-Aminolevulinic acid	PPDF	Photosynthetic photon flux density
Chl.	Chlorophyll content	PSII	Photosystem II
C _i	Intercellular concentration of CO ₂	Q _A	Plastoquinone A
CS _m	Leaf cross section	Q _B	Plastoquinone B
DI ₀ /CS _m	Energy amount dissipated from PSII	RC/CS _m	Number of active reaction centers
DW	Dry weight	RWC	Relative water content
E	Transpiration	Si	Silicon
ET ₀ /CS _m	Amount of energy used for electron transport	TRIA	Triacontanol
F _v /F _m	Quantum yield of PSII	TR0/CS _m	Amount of excitation energy trapped in PSII reaction centers
FW	Fresh weight	TW	Turgid weight
gs	Stomatal conductance	WUE	Water use efficiency
Kr	Water hydraulic conductance	ZEN	Zearalenone
PAR	Photosynthetically active radiation	φE ₀	Effective quantum yield of electron transport flux from the primary quinone acceptor Q _A to Q _B
PI	Overall performance index of PSII photochemistry	Ψ ₀	Osmotic potential

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✉ Agnieszka Ostrowska
a.ostrowska@ifr-pan.edu.pl

¹ Polish Academy of Sciences, The Franciszek Górski Institute of Plant Physiology, Niezapominajek 21, 30-239 Kraków, Poland

Introduction

Plant growth and development stimulators play an increasingly important role in global agriculture (Duan et al. 2006; Hajam et al. 2018). Contrary to traditional

fertilizers, they are used at very low concentrations (mg L^{-1}), so they do not evoke harmful or permanent environmental changes. Plant growth stimulators include, e.g., aminolevulinic acid (5-ALA), triacantanol (TRIA), zeaxalenone (ZEN), and silicon (Si). Despite different chemical structure, their common feature is the possibility of limiting negative effects of environmental stresses on crop productivity (Hotta et al. 1997; Korndorfer and Lepsch 2001; Naeem et al. 2009; Biesaga-Kościelniak and Filek 2010).

5-aminolevulinic acid (5-ALA) is an essential precursor of all porphyrin compounds, including chlorophyll and heme (von Wettstein et al. 1995; Wu et al. 2019). Exogenous application of 5-ALA diminishes negative effects of drought (Al-Khateeb 2006; Ostrowska et al. 2019), cold (Korkmaz and Korkmaz 2009), shade (Sun et al. 2009), salinity (Nishihara et al. 2003), or heavy metals (Ali et al. 2013). The stimulator affects also chlorophyll content (Xu et al. 2010), gas exchange (Akram et al. 2018), water relations (Korkmaz et al. 2010), and activity of antioxidant enzymes (Balestrasse et al. 2010; Naeem et al. 2011).

Zearalenone (ZEN) is a mycotoxin of strong estrogenic activity produced by fungi of the genus *Fusarium* (Stob et al. 1962). ZEN activity is similar to that of plant hormones (Biesaga-Kościelniak and Filek 2010; Filek et al. 2010). It influences plant yield, photosynthetic apparatus activity, fatty acid level, phytosterol content, and ion uptake (Biesaga-Kościelniak 2001; Kościelniak et al. 2009). ZEN may also change the intensity of plant respiration and CO_2 assimilation (Biesaga-Kościelniak et al. 2003; Szechyńska-Hebda et al. 2007; Biesaga-Kościelniak and Filek 2010), as well as permeability of cell membranes to electrolytes (Biesaga-Kościelniak 2001). Its regulatory activity is evidenced by the fact that it can also partly replace the low-temperature requirement for ear development in winter wheat (Fu and Meng 1994; Fu et al. 2000; Filek et al. 2010). Kościelniak et al. (2011) demonstrated a protective effect of ZEN on PSII photochemical activity and growth in wheat and soybean exposed to salt stress.

Triacantanol (TRIA), a saturated long-chain alcohol, is a natural component of plant epicuticular waxes (Ries et al. 1977; Chen et al. 2002). Exogenous application of TRIA enhances the content of photosynthetic pigments, carbohydrates, amino acids and proteins, increases dry weight and leaf area, and improves membrane integrity (Ries and Stutte 1985; Rajasekaran and Blake 1999; Muthuchelian et al. 2001). Foliar treatment with TRIA increased catalase and peroxidase activity and decreased the contents of H_2O_2 , malondialdehyde, phenolics and glycinebetaine under stress (Perveen et al. 2016).

TRIA upregulated photosynthesis and suppressed stress in rice (Chen et al. 2002). It also exerted protective effects in plants exposed to heavy metals (Muthuchelian et al. 2001),

drought (Rajasekaran and Blake 1999), or salinity (Shahbaz et al. 2013).

Although silicon (Si) is not an essential element in plants, multiple studies confirmed its positive influence on plant growth and development (Ma et al. 2006; Gunes et al. 2007; Balakhnina et al. 2012). Its protective effects during salt stress manifest in enhanced activity of antioxidant enzymes, elevated chlorophyll content and increased photochemical efficiency of PSII (Al-aghabary et al. 2005). Another study on salt stress demonstrated that an addition of silicon could increase net photosynthetic rate and reduce membrane permeability (Liang et al. 1996). Moussa (2006) showed that Si addition during stress can significantly reduce H_2O_2 content, proline level, malondialdehyde concentration, and enhance chlorophyll content and photosynthetic activity. Silicon also improved water status and increased dry mass of wheat plants under drought stress (Gong et al. 2003). Even though silicon is the second most prevalent element in the soil, majority of its sources are insoluble and occur in plant-unavailable form (Balakhnina and Borkowska 2013).

Drought stress is one of the key factors limiting crop productivity. This is a particularly challenging problem for thermophilic plants, such as maize, which are often grown in the areas experiencing precipitation shortage (Campos et al. 2004; Tsonev et al. 2009; Ghahfarokhi et al. 2015). Therefore, the aim of the study was to analyze the effects of 5-ALA, ZEN, TRIA and Si on water management and photosynthetic activity of maize under soil drought. We assumed that the exogenous application of the plant growth stimulators may alleviate the negative effects of leaf dehydration.

Materials and methods

Plant material and plant growth conditions

The experiments involved a cultivar ‘Kosmo 230’ (Małopolska Plant Breeding Station Ltd.) intended for both silage and grain yield. The cultivar is grown in water-rich soils. The experiments were carried out under controlled greenhouse conditions of 16 h photoperiod, temperature $25/20\text{ }^{\circ}\text{C}$ (day/night) and air humidity $45 \pm 5\%$. Photosynthetic photon flux density (PPDF) at the level of top leaves was about $290\text{--}380\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$. These conditions were also continued during drought.

Growth stimulators

Based on the data found in the literature, we used the following concentrations of the growth stimulators: ZEN 2 mg dm^{-3} , 5-ALA 3 mg dm^{-3} , TRIA 1 mg dm^{-3} . Silicon was applied as a commercial preparation Optysil (Intermag Ltd., Poland) that included the element in the form of

stabilized silicic acids. A solution was prepared according to the manufacturer's recommendations, in which the concentration of silicon reached 0.5 mg dm^{-3} .

Drought and treatment with growth stimulators

Experiment 1

The aim of this experiment was to determine the effect of plant growth stimulators applied at a very early stage of the plant development (three-leaf stage) under soil drought.

The plants were grown separately in pots (0.1 L) filled with vermiculite and watered once a week with Hoagland medium (Hoagland 1948). Once the plants developed their third leaf, they were divided into six groups: control without spraying (C_a), water spraying (C_w), ZEN, TRIA, 5-ALA, and silicon preparation (Si). The spraying was performed three times at two-day intervals. After the last spraying, watering was ceased until soil moisture content dropped to 35–40%, and this level was maintained for 7 days. All experimental variants (also control) were subjected to soil drought. After this time, chlorophyll content ($n=15$), plant height ($n=15$) and relative water content (RWC) ($n=7$) were measured. The measurements involved the first (Chl.) and the second (RWC) fully developed leaf from the top of a single plant. One repetition means one plant.

Experiment 2

The aim of this experiment was to determine the effect of plant growth stimulators applied at stem elongation stage on the plant development under soil drought.

Individual plants were grown in pots (2 L) filled with a mixture of garden soil and sand (2:1, v/v), and watered once a week with Hoagland medium (Hoagland 1948). At the beginning of stem elongation stage, the plants were divided into five groups, similarly as in Experiment 1 (except for C_w , no effects of spraying with water vs. C_a). Spraying with the preparations occurred three times at two-day intervals. Then, watering was stopped until the soil moisture content dropped to 35–40%, and this level was maintained for 10 days. All groups of plants (also control) were subjected to soil drought. It was controlled by weighing the pots and subtracting the plant weight. Measurements were performed on the 10th day of drought.

After this time, relative water content (RWC) ($n=7$), osmotic potential (Ψ_o) ($n=7$), chlorophyll content (Chl.) ($n=10$), intensity of stomatal conductance (g_s) ($n=10$), and water hydraulic conductance (Kr) ($n=7$) were evaluated. The measurements were performed in the first (Chl., g_s , Ψ_o) and the second (RWC) fully developed leaf from the top of a single plant. A single plant (without the stem cut off at a

height of 5 cm) was used to measure the hydraulic conductivity. One repetition means one plant.

Experiment 3

The aim of this experiment was to determine the effect of plant growth stimulators applied at heading stage on the plant development under soil drought.

Individual plants were grown in pots (4 L) filled with a mixture of garden soil and sand (2:1, v/v), and watered once a week with Hoagland medium (Hoagland 1948). Once the head emerged, the plants were divided into five variants, as in Experiment 2. The spraying with preparations was performed three times at 2 day intervals. Then, watering was stopped until the soil moisture level was 35–40%, and this level was maintained for 14 days. All groups of plants (also control) were subjected to soil drought. It was controlled by weighing the pots and subtracting the plant weight. Measurements were carried out on 14th day of drought. After this time, gas exchange ($n=7$) and photosynthetic activity ($n=12$) were assessed. Both measurements were performed in the first fully developed leaf from the top of a single plant. One repetition means one plant.

Measurements

The measurements covered three developmental stages of maize, i.e., three leaves, stem elongation and heading. For each developmental stage water relations were analyzed (three-leaf stage: RWC; stem elongation: RWC, osmotic potential, stomatal conductance, root hydraulic conductance; heading: stomatal conductance, water use efficiency), and plant productivity metrics were assessed (three-leaf stage: chlorophyll content, plant growth analysis; stem elongation: chlorophyll content; heading: activity of photosynthetic apparatus, gas exchange).

Gas exchange at the leaf level

Gas exchange was measured using an infrared gas analyzer LCpro-SD (ADC BioScientific Ltd., UK). The following parameters were measured: Photosynthetic rate (P_N), transpiration (E), stomatal conductance (g_s) as well as intercellular concentration of CO_2 (C_i). WUE was calculated as P_N/E . In chamber, CO_2 concentration was equal to $360 \mu\text{mol CO}_2 \text{ mol}^{-1}$ air, humidity was as ambient condition, PAR intensity equal to $600 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and temperature $+22^\circ\text{C}$. The adaptation of leaves to chamber condition was continued until stable gas exchange rate was observed. After this time, an automatic record of all tested parameters was made. The measurements were carried out between 10:00 a.m. and 12:00 p.m. and involved the first fully expanded leaf (Gadzinowska et al. 2019).

Stomatal conductance (g_s)

A porometer (Sc-1 Porometer; Decagon devices Inc., USA) was used to measure g_s . The measurements involved the first fully expanded leaves and were carried out between 10:00 a.m. and 12:00 p.m.

Photochemical efficiency

Photochemical efficiency was measured using a Handy PEA chlorophyll fluorimeter (Hansatech Ltd., Kings Lynn, UK). Measurements were taken after 30 min. of leaf adaptation to darkness. Light intensity reaching the leaf was $3000 \mu\text{mol (quantum)} \text{ m}^{-2} \text{ s}^{-1}$ (peak at 650 nm). Changes in fluorescence were registered during irradiation between 10 μs and 1 s. During the first 2 ms, data were gathered every 10 μs . After this time, the periodicity of measurements dropped automatically. The collected data were analyzed with a JIP test, based on the theory of energy flow in PSII (Srivastava and Strasser 1977; Lazár 1999; Strasser et al. 2000; Appenroth et al. 2001). The following parameters were calculated per CS_m : ABS/CS_m , TR_0/CS_m , ET_0/CS_m , DI_0/CS_m and RC/CS_m (Rapacz et al. 2010). Additionally, F_v/F_m , ϕE_0 and PI were determined (Ostrowska et al. 2019). The measurements involved the central part of the first completely expanded leaf.

Chlorophyll content

Measurements were performed in the first completely developed leaf from the top with a hand-held chlorophyll meter SPAD-502 (Konica-Minolta, Japan).

Relative water content (RWC)

Measurements involved the second completely expanded leaf from the top. RWC was determined according to formula: $\text{RWC} = [(FW - DW)/(TW - DW)] \times 100\%$, where FW represents fresh weight—determined immediately after cutting the leaf, TW turgid weight—determined after placed leaves for 24 h in vials containing water, DW represents dry weight—measured after 48 h drying the leaves at 80°C (Barrs and Weatherley 1962).

Leaf osmotic potential (Ψ_o)

Leaf osmotic potential was analyzed with a psychrometer HR 33 T connected with sample chambers C-52 (WESCOR, Inc., Logan, Utah, USA). The filter paper discs ($\varnothing 5 \text{ mm}$) were soaked with cell sap squeezed with a syringe from the collected leaves. Then, the disk was placed in the measuring chamber and left for 30 min. After that the measurements were taken in the dew point mode (Hura et al. 2012, 2017).

Root hydraulic conductance (K_r)

K_r was estimated using a High-Pressure Flow Meter (Dynamax Inc., USA) as described by Tyree et al. (1995). Entire intact roots were connected to the meter through the shoot excised 5 cm above the root collar and water was perfused into the root system opposite to the normal direction of flow during transpiration. The root systems were pressurized to 0.15 MPa. The perfusion pressure changed at a constant rate of $3\text{--}7 \text{ kPa s}^{-1}$, while measuring the flow into the root every 2–4 s.

Statistical analysis

Duncan's multiple range test at $p=0.05$ was performed to determine the significance of differences between treatments. All data were analyzed using Statistica 10.0 software (Statsoft Inc., USA).

Results

The applied substances improved the condition of plants exposed to soil drought at all investigated developmental stages.

The effects of silicon preparation were the most perceptible at the three-leaf stage. The plants treated with silicon demonstrated higher hydration level and chlorophyll content vs. control. We also noted positive effects of the element on plant growth (Table 1).

Treatment with TRIA and ZEN resulted in significant increase in RWC as compared with both control variants (C_a , C_w) (Table 1). Better hydration of plants treated with TRIA was associated with significant increase in plant height. The positive effects of 5-ALA were expressed by enhanced chlorophyll content in maize leaves, but the stimulator did not affect leaf water content or elongation growth (Table 1).

Treatment with 5-ALA at the stem elongation stage during soil drought increased chlorophyll content (Table 2). At this developmental stage, we also observed a significant

Table 1 Effects of the investigated stimulators on leaf relative water content (RWC), chlorophyll content (Chl.), and plant height at three-leaf stage stage (Exp.1)

Type of spraying before drought	RWC [%]	Chl [c.u.]	Plant height [cm]
C_a	83.4 b	34.6 b	21.9 b
C_w	82.2 b	33.8 b	22.4 b
5-ALA	85.0 ab	39.1 a	23.0 ab
TRIA	87.6 a	35.5 ab	25.6 a
ZEN	86.5 a	37.2 ab	23.4 ab
Silicon	87.4 a	38.8 a	25.9 a

Table 2 Effects of the investigated stimulators on relative water content (RWC) ($n=7$), osmotic potential (Ψ_o) ($n=7$), chlorophyll content (Chl.) ($n=10$), intensity of stomatal conductance (gs) ($n=10$),and water hydraulic conductance (K_r) ($n=7$) in maize roots during moderate soil drought at the stem elongation stage (Exp.2)

Type of spraying before drought	RWC [%]	Ψ_o [MPa]	Chl [c.u.]	gs [mmol (CO ₂) m ⁻² s ⁻¹]	K_r [kg s ⁻¹ MPa ⁻¹]
C _a	72.8 b	-2.34 b	41.4 b	67.21 b	8.53·10 ⁻⁶ a
5-ALA	75.0 ab	-1.95 ab	48.3 a	70.82 ab	8.86·10 ⁻⁶ a
TRIA	74.0 ab	-2.54 b	40.7 b	64.05 b	9.23·10 ⁻⁶ a
ZEN	76.8 a	-1.56 a	45.3 ab	92.69 a	6.86·10 ⁻⁶ a
Silicon	76.4 a	-1.76 a	43.9 ab	85.97 a	7.97·10 ⁻⁶ a

Table 3 Effects of the investigated stimulators on photosynthetic rate (P_N), transpiration (E), stomatal conductance (gs), intercellular concentration of CO₂ (Ci) and water use efficiency index (WUE) in maize at the heading stage ($n=7$) (Exp.3)

Type of spraying before drought	P_N	E	gs	Ci	WUE
C _a	17.4 b	1.86 c	84.2 b	128.4 ab	9.35 a
5-ALA	20.1 a	3.01 a	93.6 a	137.3 a	6.68 b
TRIA	18.0 ab	2.75 b	79.4 b	119.6 b	6.55 b
ZEN	19.7 a	2.46 b	92.1 a	118.4 b	8.01 a
Silicon	18.6 ab	2.95 ab	80.9 b	123.0 ab	6.31 b

P_N : [$\mu\text{mol (CO}_2\text{) m}^{-2}\text{ s}^{-1}$]. E: [$\text{mmol (H}_2\text{O) m}^{-2}\text{ s}^{-1}$]. gs: [$\text{mmol (H}_2\text{O) m}^{-2}\text{ s}^{-1}$]. Ci: [$\mu\text{mol (CO}_2\text{) mol (air)}^{-1}$]. WUE: [$\mu\text{mol (CO}_2\text{) mmol (H}_2\text{O)}$]

increase of leaf water content due to ZEN and Si application. In both cases, this was accompanied by considerable growth of the osmotic potential and stomatal conductance in relation to the control. TRIA treatment did not significantly affect the investigated parameters. Similarly, root hydraulic conductance remained unaffected by any of the growth stimulators (Table 2).

At maize heading stage, 5-ALA improved gas exchange parameters and photochemical activity of PSII (Table 3, Table 4). As compared with control, 5-ALA significantly enhanced net photosynthesis rate, transpiration and stomatal

conductance. At the same time, a clear reduction in water use efficiency (WUE) occurred (Table 3). The plants treated with 5-ALA showed higher activity of the photosynthetic apparatus manifested in increased values of Fv/Fm (quantum yield of PSII), PI (overall performance index of PSII photochemistry), ABS/CS_m (light energy absorption), TR_o/CS_m (amount of excitation energy trapped in PSII reaction centers) and ET_o/CS_m (amount of energy used for electron transport). This stimulator did not alter DI_o/CS_m value (energy amount dissipated from PSII) (Table 4).

ZEN application at the heading stage significantly improved net photosynthesis rate, transpiration and stomatal conductance. Intercellular concentration of CO₂ (Ci) and WUE remained unaffected (Table 3). The presence of this stimulator during soil drought significantly influenced PSII activity. We noted a boost in quantum yield of PSII, overall performance index of PSII photochemistry and the amount of energy used for electron transport, while ABS/CS_m and TR_o/CS_m remained unaltered.

At the heading stage occurring during soil drought TRIA influence on gas exchange was less perceptible and manifested in significant increase of transpiration intensity as compared with control. The influence was accompanied by reduced water use efficiency (WUE) (Table 3). Treatment with TRIA significantly enhanced light energy absorption (ABS/CS_m) and the amount of energy used for electron transport (ET_o/CS_m) together with DI_o/CS_m value (Table 4).

Table 4 Effects of the investigated stimulators on quantum yield of PSII (F_v/F_m), effective quantum yield of electron transport flux from the primary quinone acceptor Q_A to Q_B (ϕE_o) and overall performance index of PSII photochemistry (PI) and also parameters of JIP test: light energy absorption (ABS/CS_m), amount of excitation energytrapped in PSII reaction centers (TR_o/CS_m), amount of energy used for electron transport (ET_o/CS_m), energy amount dissipated from PSII (DI_o/CS_m) and number of active reaction centers (RC/CS_m) per excited leaf cross-section (CS_m) in maize at the heading stage ($n=12$) (Exp.3)

Type of spraying before drought	F _v /F _m	ϕE_o	PI	ABS/CS _m	TR _o /CS _m	ET _o /CS _m	DI _o /CS _m	RC/CS _m
C _a	0.701 b	0.34 a	0.85 c	2023.6 c	1374.1 b	694.3 c	649.5 b	628.4 a
5-ALA	0.758 a	0.33 a	0.96 a	2253.4 a	1567.7 a	743.8 a	685.7 b	662.8 a
TRIA	0.714 b	0.34 a	0.87 bc	2174.0 b	1415.2 ab	737.9 ab	758.8 a	647.3 a
ZEN	0.762 a	0.36 a	0.91 ab	2049.2 c	1410.0 ab	729.1 ab	639.2 b	627.9 a
Silicon	0.737 ab	0.35 a	0.89 bc	2075.4 c	1354.1 b	717.4 bc	721.3 a	690.8 a

Silicon preparation applied at the heading stage intensified transpiration and reduced WUE (Table 3). Its effects on the photosynthetic apparatus included a significant increase in the amount of energy dissipated from PSII (DI_o/CS_m) (Table 4).

None of the experimental growth stimulators triggered changes in the number of active reaction centers (RC/CS_m), effective quantum yield of electron transport flux from the primary quinone acceptor Q_A to Q_B (ϕE_o) or intercellular concentration of CO_2 (C_i) (Tables 3, 4). The parameter most sensitive to their application was transpiration intensity (Table 3), and among chlorophyll fluorescence parameters the one associated with the amount of energy used for electron transport (ET_o/CS_m) (Table 4).

Discussion

Our experiments showed a modification of maize response to soil drought triggered by the application of plant growth stimulators. All the investigated substances affected water relations and photosynthetic activity with different intensity and distinctive effects.

5-ALA influenced mainly gas exchange and photochemical activity of PSII (Tables 3, 4). A similar response of the photosynthetic apparatus to 5-ALA was reported for salinity stress (Naeem et al. 2011) and excessive presence of cadmium (Ali et al. 2013) or chromium (Ahmad et al. 2017). Our study demonstrated also a stimulatory effect of 5-ALA on transpiration intensity. On one hand, high transpiration intensity and a boost in stomatal conductance observed during drought stress may result in rapid plant dehydration, but on the other hand, they allow for maintaining gas exchange. Lowered photosynthesis rate during drought is mainly due to stomatal closure that limits availability of CO_2 (Mansfield and Davies 1981). Other researchers reported the influence of 5-ALA on energy flow in thylakoid membranes (Ali et al. 2013; Liu et al. 2016) and quantum yield of PSII (F_v/F_m) (Akram et al. 2012; Wang et al. 2018). Another positive effect of 5-ALA application included a boost in chlorophyll content under drought (Table 1, Table 2), which was probably due to the fact that 5-aminolevulinic acid serves as a precursor in chlorophyll biosynthesis (Akram and Ashraf 2013; Kosar et al. 2015; Ahmad et al. 2017; Akram et al. 2018).

We observed similar changes regarding photosynthesis, transpiration and stomatal conductance following the application of ZEN in plants exposed to soil drought (Table 3). These effects are concurrent with those reported by Kościelniak et al. (2009), who demonstrated a stimulatory effect of ZEN (pre-sowing seed soaking in the stimulator solution) on gas exchange in wheat at the stage of young ears visible and in soybean during rapid pod growth. However,

they did not report an increase in the quantum yield of PSII (F_v/F_m). Kościelniak et al. (2009) proved that soaking wheat leaf discs for 48 h in the solutions of ZEN of various concentrations lowered the values of three parameters associated with light energy absorption (ABS/CS), amount of excitation energy trapped in PSII reaction centers (TR_o/CS_m), and the amount of energy used for electron transport (ET_o/CS_m). Opposite effects for the same chlorophyll fluorescence parameters were reported for soybean (Kościelniak et al. 2011).

TRIA influence on maize condition was clearly visible at the seedling stage and included stimulation of water relations and elongation growth. Similar effect was observed in tomato, hyacinth bean and sweet basil (Khan et al. 2009; Naeem et al. 2009; Hashmi et al. 2011). Thakur et al. (1998) described the favorable effect of TRIA on leaf water content in olive plants exposed to soil drought. Reports on positive effects of TRIA on chlorophyll content and photochemical activity of PSII (Kumaravelu et al. 2000; Chen et al. 2002, 2003) were not clearly confirmed in our study, particularly regarding chlorophyll content (Tables 1, 2, Table 4). Similarly, to Eriksen et al. (1981), we showed no influence of TRIA on net photosynthesis in maize, even though this was previously reported for other plant species (Misra and Srivastava 1991; Naeem et al. 2009). Lack of TRIA impact on photosynthesis was unexpected, as the compound induces the activity of many genes involved in the process (Chen et al. 2002, 2003). Foliar application of L (+)-adenosine precursor in TRIA biosynthesis in maize, cucumber and tomato may induce changes in the level of Ca^{2+} , Mg^{2+} and K^+ in the exudates obtained from the stumps (Ries et al. 1993). Plant response to TRIA depends on many factors, including species, developmental stage, stimulator dose, application manner, number of treatments, growth conditions and stress intensity (Eriksen et al. 1982).

The response to silicon preparation in plants exposed to soil drought differed based on developmental stages. Though silicon is still not considered an element essential for higher plants, its advantageous effects have been demonstrated for many species, especially under biotic or abiotic stress conditions (Ma et al. 2006; Liang et al. 2007; Etesami and Jeong 2018; Sirisuntornlak et al. 2019). The positive effects of silicon we observed in maize during drought stress (Table 1) were also reported in other cereals, i.e., sorghum and wheat (Gong et al. 2003; Hattori et al. 2005). Greater intensity of transpiration induced by this stress in maize (Table 3) corroborated similar results published by Hattori et al. (2005) for sorghum, and was accompanied by an increase in photosynthesis rate and stomatal conductance. Other authors speculated on a link between this effect and stimulation of root system development by Si and resulting enhancement of water uptake ability. In maize, silicon application during water stress did not alter root hydraulic conductance

(Table 2). However, the element significantly influenced plant water status at earlier stages of three leaves (Table 1) and stem elongation (Table 2). This was manifested in higher RWC, and in the case of stem elongation stage also in higher osmotic potential (Table 2). The silicon preparation noticeably affected chlorophyll content at the stage of three leaves. In wheat leaves, Si application enhanced the contents of chlorophyll *a*, chlorophyll *b*, and carotenoids during cadmium stress (Hussain et al. 2015).

In summary, the impact of the investigated substances during drought stress depended on maize developmental stage. 5-ALA induced changes in photosynthesis by affecting chlorophyll level, gas exchange and photochemical activity of PSII. ZEN influence was highly similar to that of 5-ALA but apart from stimulating gas exchange and photochemical activity of PSII, it also induced stem elongation and limited plant dehydration. Beneficial effects of TRIA were the most perceptible at the stage of three leaves and involved leaf hydration and plant growth during drought. Silicon preparation triggered a response similar to TRIA at the stage of three leaves. Apart from improving water relations and plant growth, Si altered also chlorophyll levels. All the stimulators significantly affected transpiration intensity at the heading stage.

Author contribution statement AO carried out the experiment, data analysis and the preparation of manuscript. TH performed critical revision of the manuscript. MTG and TH participated in biochemical analysis. All authors read and approved the final manuscript.

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Declarations

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

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