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Full Length Article

Light Augments the Action of Foliar Applied Plant Growth Regulators: Evidence using Etiolated Maize (*Zea mays*) Seedlings

Shazia Batool[1*], Abdul Wahid[1], Shahzad M.A. Basra[2] and Muhammad Shahbaz[1]
[1]*Department of Botany, University of Agriculture, Faisalabad 38040, Pakistan*
[2]*Department of Agronomy, University of Agriculture, Faisalabad 38040, Pakistan*
[*]For correspondence: drawahid@uaf.edu.pk; drawahid2001@yahoo.com
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Abstract

Reduced availability or absence of light is a major debacle for plant growth and yield. Exogenous application of plant growth regulators (PGRs) partially replaces light requirement(s) during seed germination, but studies are scarce on the interactive role of light and the PGRs in etiolated plants. In this research, non-etiolated, etiolated and de-etiolated maize (*Zea mays* L.) hybrid P-1543 seedlings were sprayed with optimized levels of ascorbic acid (AsA; 0.5 m*M*), thiourea (TU; 10 m*M*), cycocel (CCC; 1.2 m*M*) and kinetin (KIN; 3.0 μM) and grown for six days. Etiolation caused seedling elongation, reduced dry weight but increased elongation-to-dry weight ratio, which decreased strongly in de-etiolated seedlings. Reductions in chlorophyll (*Chl*) *a* and *b*, carotenoids (*Car*), net photosynthesis (*Pn*), transpiration rate (*E*) and stomatal conductance (*gs*) under etiolation (27–48%) were lowly improved with the PGRs under no-etiolation (5–15%), but were greatly improved (18–76%) in de-etiolated seedlings. Etiolation exacerbated the hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents, which were marginally lowered with the PGRs spray (18 and 12%, respectively). Nonetheless in de-etiolated seedlings, the PGRs spray declined H_2O_2 (32%) and MDA (40%) contents apropos improving soluble phenolics (PHE), flavonoids (FLA), anthocyanins (ANT), ascorbate (AsA), niacin (NIA) and riboflavin (RIB) contents (20–37%). Although not correlated in etiolated seedlings, the seedling dry weight was positively associated with pigments, leaf gas exchange, the PHE and ANT, but negatively with substomatal CO_2 concentration (*Ci*) of de-etiolated seedlings. The applied PGRs alleviated oxidative damage, improved ANT, FLA, pigment contents and leaf gas exchange in aptly producing stout seedlings during de-etiolation. In conclusion, the hypothesis that light was quite effective in augmenting the effects of PGRs in seedling growth, which were principally due to alleviation of oxidative damage and improved photosynthesis was accepted. © 2020 Friends Science Publishers

Keywords: De-etiolation; Oxidative damage; Light; Correlation; Maize; PGRs

Introduction

During their life-span, land plants, being sessile, have to face sporadic climatic perturbations (Becklin *et al.* 2016). Light is a factor of key importance as almost all the biological phenomena in plant are directly or indirectly rely upon light. A mild to severe decline in the available light is amongst the critical limiting factors for photosynthesis and plant growth. Under sub-due light the plants dramatically change their morphogenetic pattern by portraying etiolation (Svriz *et al.* 2014; Tiryaki and Kaplan 2019). The etiolated plants show long hypocotyls, week cell walls and extensive loss of photosynthetic pigments, especially the chlorophylls with the conversion of chloroplasts to etioplasts (Liu *et al.* 2017). This underlines that etiolation is a sub-optimal condition, arising from low- or non-availability of light, initiating an array of molecular and physiological changes and leading eventually to a ceased pigment synthesis and weakening of the cell wall of internodes (Sinclair *et al.* 2017).

The PGRs are of pivotal importance in triggering key processes in plants (Symons and Reid 2003). In agricultural practices, the growth promoters and retarders are used to improve crop productivity when applied via different modes. The exogenous application of PGRs is effective in enhancing the crop growth and productivity under optimal and sub-optimal conditions. Among five naturally occurring PGRs, gibberellins mobilize the seed reserves during germination, promote elongation growth of stem, and trigger transition from vegetative to reproductive growth (Binenbaum *et al.* 2018). Auxins signal the plant processes like cell expansion, root branching and fruit development (Petrasek *et al.* 2019). The cytokinins promote the cell division in the meristematic tissues, delay senescence, and promote chlorophyll synthesis (Klíčová *et al.* 2004; Petrasek *et al.* 2019). Abscisic acid closes the stomata, induces seed and bud dormancy and hastens senescence (Tardieu *et al.* 2010). Gaseous hormone ethylene controls the ripening of climacteric fruits, floral induction, plant sex determination

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and promotion of abscission (Klíčová *et al.* 2004; Kabir *et al.* 2018). In addition, there are some recently introduced PGRs and chemical substances, which modulate an array of plant processes. For instance, brassinosteroids control cell elongation, gravitropism, maintain apical meristem and root hair differentiation (Wei and Li 2016; Peres *et al.* 2019). Jasmonates play roles in wound response and herbivory resistance (Katsir *et al.* 2008; Koo and Howe 2009). Similarly, strigolactone promote the seminal and adventitious root formation (Sun *et al.* 2016). At the same time, triacantonol promotes photosynthesis, proteins synthesis, water and nutrient uptake (Naeem *et al.* 2012; Sharma *et al.* 2018).

One of the main aspects of light and growth regulators in plants is the elicitation of morphogenetic responses with the activation of the photoreceptors and their interacting factors (*e.g.*, phytochrome interacting factors; PIFs) during seed germination and seedling growth (Lau and Deng, 2010; Pham *et al.* 2018). Studies show that there is interplay between light and hormone signaling pathways for the chlorophyll biosynthesis during the etiolation-de-etiolation transitions (Liu *et al.* 2017). The proteins such as *PIFs*, *ELONGATED HYPOCOTYL 5*, *ETHYLENE INSENSITIVE 3* and *DELLA* are key transcriptional regulators in light and hormonal signaling pathways (Liu *et al.* 2017). Using *altered meristem program* (*Amp) 1* mutant of Arabidopsis it has been revealed that cytokinin or cytokinin-mediated processes are the regulators of etiolation response by acting as a component of the induction of morphogenetic processes via signal transduction pathway, which may be independent of light (ChinAtkins *et al.* 1996). During the search for mutants showing a synergistic hormonal stimulatory response, a novel allele 7 of *amp1* (*amp1-7*) mutant was found to show coactive stimulatory effect of ethylene and gibberellins (Saibo *et al.* 2007).

It is evident from the above that both light and PGRs intimately interact with each other in modifying the plant morphogenetic responses during seed germination and seedling growth. While the exogenous application of PGRs can partially replace the requirement of light during seed germination (Sawada *et al.* 2008; Miransari and Smith 2014), there is no report of the hormonal regulation in etiolated seedlings. To test the hypothesis that etiolation is a modifying factor and that the foliar spray of PGRs may mitigate the negative effects of etiolation by producing profound changes, this study was performed to explore the possible bio-regulatory role of foliar spray of optimized levels of selected PGRs including ascorbic acid (AsA), thiourea (TU), cycocel (CCC) and kinetin (Kin) in the non-etiolated, etiolated and de-etiolated maize seedlings based on an array of growth and physiological attributes.

Materials and Methods

Plant material and growth conditions

Maize (*Zea mays* L. Hybrid P-1543) caryopses were obtained from Poineer Seed Co., Sahiwal, Pakistan. The experiments were conducted in the growth room facility of the Department of Botany, University of Agriculture, Faisalabad, Pakistan. Ten seeds were sown in plastic pots (20 cm long, 40 and 30 cm diameter at the top and bottom, respectively). Each pot contained 2 kg of the washed sand. The caryopses were surface sterilized with 35% H_2O_2 for 15 min followed by thrice rinsing with sterilized water, and sown after adding water in the pots (60–70% moisture). Three days after germination, the seedlings were supplemented with 250 mL per pot of half-strength nutrient solution (Hoagland and Arnon 1950). The uniform sized seedlings were grown for six days in each pot at 350–400 µmol/m^2/s of while light (supplemented with LEDs), 60% RH and day/night temperature of 27±2°C/23±2°C and 14/10 h day/night.

Selection and optimization of the pgr levels

In this study we used four PGRs with contrasting modes of action but all these are used to improve the growth and yield of field crops. Among the foliar-sprayed PGRs, AsA acts as cofactor in the activities of different enzymes, triggers phytochrome mediated signaling (Barth *et al.* 2006; Farooq *et al.* 2013), regulates cell cycle and cell elongation (Gallie 2013; Ivanov 2014) and augments plant defense (Lu *et al.* 2019). TU, having different functional groups, exhibits growth bioregulatory effects in a number of plants both under stress and non-stress conditions (Wahid *et al.* 2017). The CCC is a synthetic growth retardant and shortens the intermodal length by reducing cell division and cell elongation, and diverting assimilates for increasing grain yield (North *et al.* 2010; Kumar and Sharma 2019). Kin improves growth and gas exchange properties (Shah 2007) and enhances chlorophyll synthesis by stimulating the 5-aminolevulinic acid synthesis (Yaronskaya *et al.* 2007).

In order to optimize the most effective levels of these PGRs, range of ascorbic acid (AsA; 0–1.25 m*M*), thiourea (TU; 0–25 m*M*), cycocel (CCC; 0–2 m*M*) and kinetin (KIN; 0–20 m*M*) levels was foliar sprayed on three days old seedlings and these seedlings were grown for six days. In addition to an unsprayed set of plants, another set of plants foliar sprayed with distilled water was run alongside as positive control. At harvest the data were recorded for seedling length, dry weight and seedling length/dry weight ratio. Optimal levels of AsA, TU, CCC and KIN were 0.5, 10, 1.2 and 3 m*M*, respectively, which were used for foliar application on non-etiolated, etiolating and de-etiolating maize seedlings.

Etiolation and de-etiolation treatments

Three sets of maize seedlings were grown in the pots to find out growth and physiological changes in the non-etiolated, etiolated and de-etiolated maize seedlings. For etiolation, before shifting to darkness, the seedlings were foliar sprayed with distilled water and pre-optimized levels of the selected

PGRs. These seedlings were grown for six days in the darkness and then measured for growth and physiological attributes. Another set of pots was running in parallel in the darkness for etiolation. These six days etiolated seedlings were foliar sprayed with the above mentioned levels of PGRs and kept in the light for de-etiolation under the above mentioned growth conditions for six days. A non-etiolated set of plants but foliar sprayed with water and PGRs for six days was run alongside for comparison. In all, there were 18 treatments; each replicated thrice with 10 uniform seedlings per replication. Experimental design was completely randomized with etiolation conditions and hormones as two factors.

Harvesting

After six days, non-etiolated, etiolated and de-etiolated maize seedlings (age 18 days) were harvested and measured for different growth and physiological attributes. The harvested seedlings were transferred to zip-lock bags, instantly frozen, and stored in a deep freezer (Sanyo, Japan) at -40^{o}C until used.

Growth, pigments and gas exchange measurements

At harvest, the seedlings were measured for length, dry weight and length to dry weight ratio. Shortly after harvest, seedlings were chopped, ground in coarse sand using 80% acetone, filtered and absorbance of the extract was taken at 490, 645 and 663 nm using spectrophotometer (Model UV-1100, Shanghai, China). The contents of *Chl a* and *b* were calculated by the method of Arnon (1949) while those of carotenoids (*Car*) was measured with Davies (1976) method. Total chlorophyll (*Chl*) contents were calculated by summing up *Chl a* and *b*, while *Chl a/b* ratio was also computed.

On the 6th day, the leaf gas exchange parameters were measured. Fully expanded leaf from the top was selected to determine net rate of photosynthesis (*Pn*), transpiration rate (*E*), stomatal conductance (g_s) and sub-stomatal CO_2 concentration (*Ci*) using Infra-red Gas Analyzer (IRGA; Model LCA 4, Analytical Development Co. Ltd., Hoddesdon, England) equipped with narrow leaf chamber. The set of conditions for these determinations was: molar air flow per unit leaf 331 m*M*/m/s, atmospheric pressure 99.8 kPa, photosynthetically active radiations on leaf surface 374 μmol/m^2/s, CO_2 concentration 388 μmol/mol and ambient temperature 25±2^{o}C. All measurements were made in triplicate.

Oxidative stress parameters

Both H_2O_2 and MDA were determined as indices of oxidative damage. For the measurement of H_2O_2 contents, with the method of Velikova *et al.* (2000), 0.1 g fresh plant material was homogenized in 1 mL of 0.1% (W/V) trichloroacetic acid (TCA) in an ice bath. After centrifuging the homogenate at 12000×*g* for 15 min, 0.5 mL of the supernatant was mixed with 0.5 mL of the phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide. After vortexing, the absorbance of the mixture was taken at 390 nm. A standard curve was prepared using an increasing series of H_2O_2 standards to determine the H_2O_2 concentration in unknown samples.

For the measurement of MDA contents, with the method of Heath and Packer (1968), 1 mL of above supernatant was mixed with I mL of 0.5% thiobarbituric acid prepared in 20% (W/V) TCA and kept in water bath preheated at 95^{o}C for 50 min. After cooling in an ice bath, absorbance of the reaction mixture was noted at 532 nm and 600 nm. For comparison 1% TCA was used as blank. The amount of MDA was calculated as:

$$\text{MDA (nmol/g fresh weight)} = [(A_{532}-A_{600})/1550000] \times 10^6.$$

Antioxidants

The analysis was done for soluble phenolics (PHE) flavonoids (FLA), anthocyanins (ANT), ascorbic acid (AsA), niacin (NIA) and riboflavin (RIB). For the estimation of PHE contents, with the method of Julkunen-Tiitto (1985), fresh plant material was extracted in 80% acetone in a water bath at 50^{o}C for 1 h. After centrifugation at 12000 × *g*, 100 μL of the supernatant was diluted with d.H_2O, mixed and added 0.5 mL of the Folin Phenol reagent and vortexed for 5 sec. The 2.5 mL of 20% Na_2CO_3 was added and vortexed for 5 sec, waited for 20 min and absorbance taken at 750 nm; 80% acetone was used as blank. For the estimation of FLA contents, 1 mL of the above acetonic extract was mixed with 4 mL water in measuring flask, mixed and added 0.6 mL of $NaNO_2$ and 0.5 of 10% $AlCl_3$ after 5 min and 2 mL of NaOH after 1 min. After adding 2.4 mL of d.H_2O, the reaction mixture was shaken well, let it stand at room temperature and measured the absorbance at 510 nm, using 80% acetone as a blank (Zhishen *et al.* 1999). For the quantification of ANT, 0.1 g of fresh material was extracted with 1 mL of acidified methanol (1% v/v HCl) at 50^{o}C in a water bath for 1 h and filtered. The absorbance of the filtrate was taken at 535 nm using acidified methanol as blank (Stark and Wray 1989).

To quantify the contents AsA in the plant samples, 0.25 g of fresh material was ground in 6% TCA and filtered. One mL of the filtrate was mixed with 1 mL of dinitrophenyl hydrazine and 1 drop of 10% TU (prepared in 70% ethanol). Boiled the mixture for 10 min, cooled in an ice bath, warmed to room temperature and added 1 mL of 80% H_2SO_4. The absorbance of the solution was taken at 530 nm using TCA as blank (Mukherjee and Choudhuri 1983). To quantify NIA with the method of Okwu and Josiah (2006), 0.1 g of the fresh sample was treated with 1 mL of 1 N H_2SO_4 for 20 min. After adding a drop of ammonia, the solution was filtered and 1 mL of filtrate was mixed with 0.5 mL of 10% of potassium cyanide solution followed by the addition of 0.5

mL of 0.02 N H_2SO_4. The reaction mixture was shaken well, let stand at room temperature and absorbance noted at 470 nm. To measure the RIB, 0.5 g of the fresh material was ground with 50% ethanol and filtered. Filtrate (1 mL) was mixed with 5% $KMnO_4$ solution and 1 mL of 30% H_2O_2, and heated the mixture at 50°C for 30 min. After cooling, 0.2 mL of Na_2SO_4 solution was added and diluted the reaction mixture to 5 mL. After 5 min the upper colorless layer was aspirated and its absorbance was measured at 510 nm. Ethanol (50%) was used as blank (Okwu and Josiah 2006).

Statistical analysis

Ten seedlings were taken per replication for the measurement of different growth and physiological attributes. Two-way analysis of variance (ANOVA) and comparison of treatment means ($P = 0.05$) were performed and treatment means were computed by applying LSD test using STATISTIX.8.1 software. Trend-lines and correlations were drawn using MS Excel (v. 2010) to find out any possible association of shoot dry weight and different physiological parameters separately for non-etiolated, etiolated and de-etiolated treatments.

Results

Seedling growth data

With non-significant ($P > 0.05$) difference among the treatments in non-etiolated seedlings, the KIN spray was the most effective (16%) in enhancing the shoot length followed by TU (15%) while CCC declined it (16%). Etiolation for six days let the seedlings to elongate and a maximum elongation was noted in water sprayed seedlings while a minimal elongation was noted with CCC spray (28%). Foliar spray of PGRs during seedling de-etiolation nearly ceased the seedlings elongation with the greatest decline in shoot length was achieved with CCC (28%) followed by KIN (12%) while water spray enhanced this attribute by 2% (Fig. 1). With significant difference in the treatments ($P < 0.01$), seedling dry weight data revealed that under non-etiolation all foliar spray treatments significantly enhanced shoot dry weight but the increase was maximum with CCC (32%) and KIN (24%) spray. Under etiolation the seedling dry weight did not differ much with foliar spray of PGRs. However, under de-etiolation, AsA and CCC were the most effective in enhancing the seedling dry weight (18% each) among all the treatments (Fig. 1). Showing significant ($P < 0.01$) difference among treatments, shoot length-to-dry weight ratio was legibly reduced with CCC spray (36% of control) followed by water spray (12%) in non-etiolated seedlings. In etiolated seedlings, this ratio was reduced highly with CCC (28%) and KIN (13%) spray. Under de-etiolation, shoot length-to-dry weight ratio was the lowest with CCC spray (37%) followed by KIN spray (19%) among all the foliar spray treatments (Fig. 1).

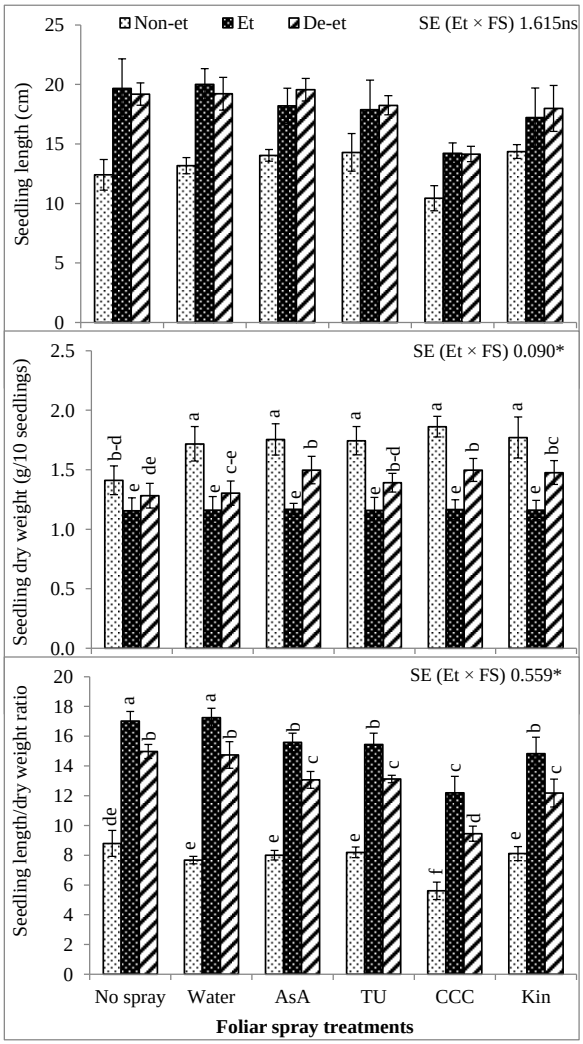

Fig. 1: Changes in growth traits of non-etiolated (Non-Et), etiolated (Et) and de-etiolated (De-Et) maize seedlings with or without the foliar spray of water and PGRs. *, $P < 0.05$); ns, $P > 0.05$. Means sharing the same letter differ non-significantly ($P > 0.05$)

Photosynthetic pigments

Although all the foliar spray treatments increased *Chl a* contents in non-etiolated plants, KIN was the most effective (14%) followed by AsA (13%). Etiolated seedlings indicated a considerable loss of *Chl a* while CCC and KIN were more promising in reducing the etiolation-induced loss of *Chl a*. In de-etiolated seedlings, however and greater *de novo* synthesis of *Chl a* was recorded with PGRs spray; KIN (44%) and CCC (42%) were more effective than the other PGRs (Fig. 2). KIN (40%) followed by AsA (34%) were more effective in improving the *Chl b* contents in non-etiolated seedlings. The *Chl b* content decline drastically in etiolated seedlings and a greatest decline (39%) was noticed in water sprayed seedlings but the least (11%) in TU treated leaves. De-etiolation recouped the *Chl b* content with all the

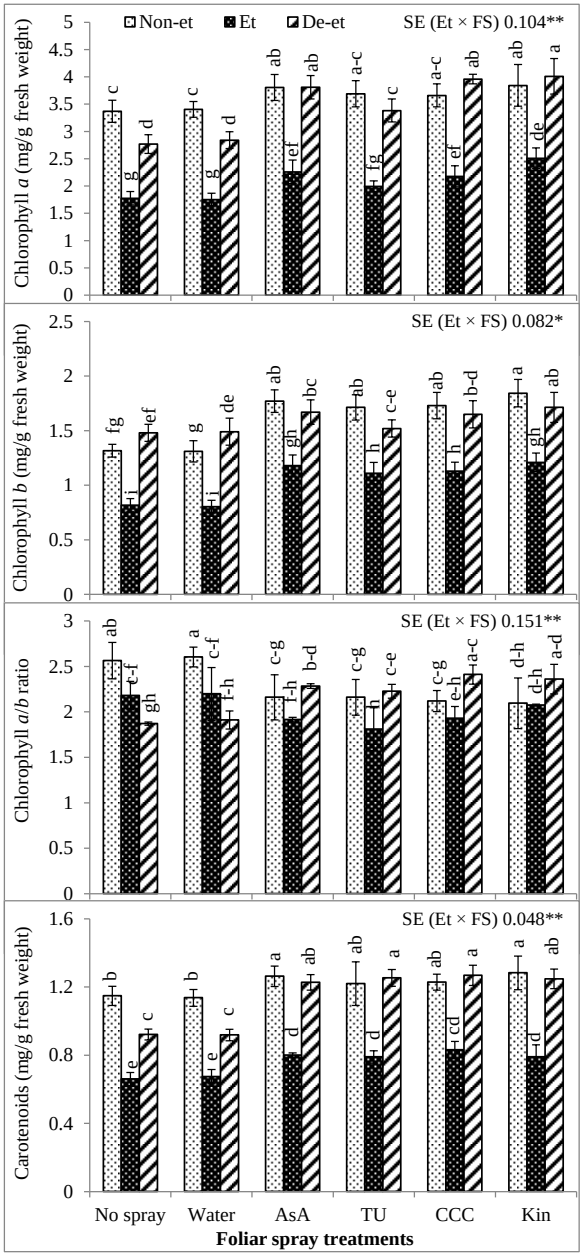

Fig. 2: Changes in photosynthetic pigment contents of non-etiolated (Non-et), etiolated (Et) and de-etiolated (Det) maize seedlings with or without the foliar spray of water and PGRs. *, $P < 0.05$); **, $P < 0.01$. Means sharing the same alphabet differ non-significantly ($P > 0.05$)

PGR treatments whereas KIN (16%) was the more effective among the PGRs (Fig. 2). In non-etiolated seedlings, *Chl a*/*b* ratio was reduced with all the foliar spray treatments. The etiolation declined this ratio irrespective of the PGRs foliar spray but a maximum decline (22%) was noted with TU spray (Fig. 2). Contrarily, in de-etiolated seedlings, there was a substantial regain in the *Chl a*/*b* ratio; being greater with CCC (29%) followed by KIN (26%) spray (Fig. 2). The *Car* contents in non-etiolated seedlings increased with all the foliar spray treatments while the KIN with 12% increase was the most effective. Under etiolation treatment, the *Car* contents declined markedly in all the treatments although foliar spray with CCC and AsA was the most effective (increased by 26 and 21%, respectively) showing a lesser decline in this attribute. However, there was a substantial increase in *Car* contents of de-etiolated seedlings in all the foliar spray treatments; nonetheless, the foliar spray of CCC was the most effective (38%) followed by KIN (35%) in improving this pigment (Fig. 2).

Gas exchange characteristics

In non-etiolated seedlings, all the PGRs spray increased *Pn*; CCC (25%) followed by AsA (23%) were more effective. Under etiolation, although there was 62% decline in *Pn* than in non-sprayed plants, the foliar spray of KIN and TU reduced this decline (44% each). Under de-etiolation, there was a gain in *Pn* approaching nearly the level of non-etiolated plants (Table 1). All the foliar spray treatments under non-etiolation improved *E*; being the highest with KIN (23%) followed by CCC (18%) spray. In etiolated seedlings, *E* declined tangibly although TU spray was more effective amongst all PGRs. De-etiolated seedlings displayed a gain in *E* with PGRs spray approaching the non-etiolated plants while KIN (with 20% increase) was highly effective (Table 1). Although all the foliar spray treatments increased g_s as compared to non-etiolated seedlings, TU and AsA (with 12 & 11% increase, respectively) were more effective. Etiolation of plants for six days, revealed a substantial decline in g_s although foliar spray of AsA (20%) and KIN (17%) was highly effective. In de-etiolated seedlings, g_s was recouped with all foliar spray treatment but KIN (22%) and CCC (20%) were more effective (Table 1). The *Ci* remained consistent across all the foliar spray treatment in non-etiolated seedlings. Etiolated seedlings indicated a large increase in this attribute, but a lower increase was produced by KIN (29%) and TU (28%). In de-etiolated seedlings, there was a noticeable decline in this attribute; while among the foliar spray treatments CCC (14% decline) and KIN (13% decline) were more effective (Table 1).

Oxidative damage parameters

In non-etiolated seedlings, the foliar spray of PGRs declined the H_2O_2 contents in maize plants with a highest decline with CCC (18%) and KIN (17%). Etiolated seedlings indicated enormously increased tissue H_2O_2 level although a least accumulation was measured in KIN (34%) and TU (33%) sprayed seedlings. In de-etiolated seedlings there was an ample decline in H_2O_2 contents but a highest decline (32%) was noted in KIN sprayed seedlings (Fig. 3). Under non-etiolated condition MDA content was reduced by ~11% with foliar spray of PGRs. Etiolated seedlings showed ~3-folds higher MDA which was distinctively reduced with PGRs spray. However, in de-etiolated

Table 1: Some gas exchange characteristics of control (non-etiolated), etiolated and de-etiolated maize seedlings with or without the foliar spray of PGRs

Etiolation (Et) treatments	Foliar spray (FS)	Net rate of photosynthesis ($\mu mol/m^2/s$)	Transpiration rate ($mmol/m^2/s$)	Stomatal conductance ($mol/m^2/s$)	Sub-stomatal CO_2 level ($\mu mol/mol$)
Non-etiolated	No spray	18.61±1.24ef	3.65±0.21d-f	0.337±0.014c	239.7±16.1
	Water	19.64±1.42de	3.67±0.26c-f	0.344±0.016bc	240.9±21.8
	AsA	22.95±1.38ab	4.10±0.21a-d	0.375±0.015a	231.1±16.1
	TU	21.09±1.52b-d	3.99±0.24b-d	0.378±0.017a	241.2±19.8
	CCC	23.35±1.33a	4.30±0.24ab	0.346±0.017bc	238.5±17.8
	Kin	22.65±1.37a-c	4.49±0.33a	0.368±0.023ab	230.2±17.2
No-et→Et	No spray	7.14±0.76i	2.65±0.26i	0.245±0.017fg	353.4±18.1
	Water	8.75±0.86hi	3.32±0.21e-h	0.225±0.015g	344.7±14.4
	AsA	9.48±0.75h	2.96±0.22h-i	0.294±0.013de	314.8±20.0
	TU	11.71±1.07g	3.26±0.22f-h	0.268±0.015ef	309.5±13.0
	CCC	12.61±1.30g	3.11±0.18gh	0.270±0.014ef	315.7±15.9
	Kin	12.58±1.14g	3.11±0.29gh	0.286±0.020e	297.8±15.9
Et→Det	No spray	17.36±1.43f	3.42±0.26e-g	0.321±0.018cd	269.9±10.1
	Water	16.85±1.07f	3.24±0.28f-h	0.328±0.019c	268.8±20.0
	AsA	20.58±1.90c-e	4.04±0.38a-d	0.369±0.014ab	238.9±11.3
	TU	20.78±1.56cd	3.72±0.43c-e	0.347±0.012bc	243.0±11.3
	CCC	21.09±1.43b-d	4.06±0.27a-d	0.385±0.016a	233.0±15.8
	Kin	21.15±1.16b-d	4.11±0.32a-c	0.393±0.018a	236.0±19.9
	SE (Et × FS)	1.056*	0.224*	0.013*	ns

*, $P < 0.05$; **, $P < 0.01$; ns, $P > 0.05$. Means sharing same alphabet differ non-significantly ($P > 0.05$)

seedlings, the foliar spray of PGRs declined MDA but a higher decline was noticed in KIN (40%) and AsA (38%) sprayed seedlings (Fig. 3).

Phenolics accumulation

In non-etiolated seedlings foliar spray of the PGRs enhanced the content of PHE; being the highest due to CCC spray (27%). Etiolated seedlings exhibited 35–50% reduction in PHE in non-sprayed and water-sprayed seedlings but foliar spray of the PGRs was quite effective in curtailing the loss of PHE; the loss was the lowest (26%) with AsA spray. In the de-etiolated seedlings, there was a regain in the PHE contents with spray of all the PGRs but CCC with 32% regain was the most promising (Table 2). The PGRs spray on non-etiolated seedlings indicated up to 19% (with CCC) accumulation of FLA. Non-sprayed etiolated seedlings suffered a large decline in FLA contents but foliar spray of growth regulators incurred a lesser decline; being the lowest (30%) with KIN spray. However, spray of the PGRs on de-etiolated seedlings recuperated the decline in FLA contents and maximum increase (22%) was achieved with foliar spray of KIN (Table 2). Foliar spray of PGRs on non-etiolated seedlings improved the ANT content, and a greatest increase (17%) was recorded with CCC spray. Etiolated seedlings indicated 41–50% decline in ANT content but the lowest loss in this metabolite was observed with AsA spray. In de-etiolated seedlings the PGRs spray legibly improved the ANT content, but CCC was the most effective (Table 2).

Vitamins accumulation

In non-etiolated seedlings, all the foliar spray of PGRs increased AsA contents while the KIN and TU (9% increase with each) were more effective. Etiolated seedlings

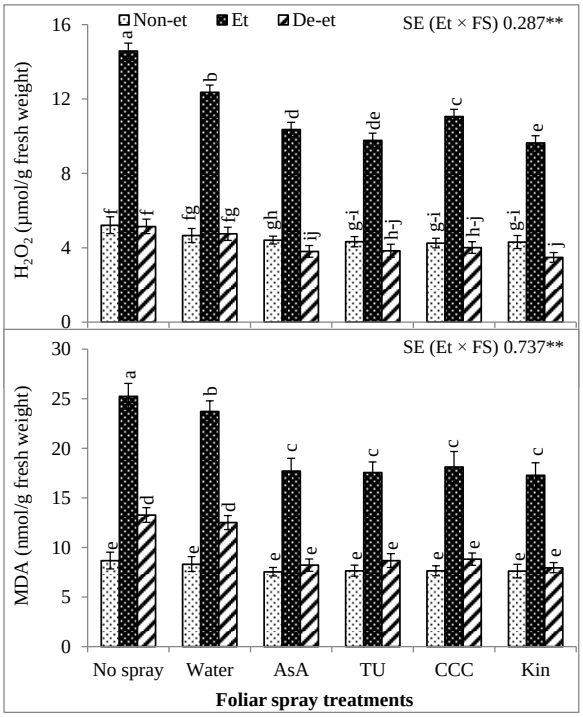

Fig. 3: Changes in the oxidative stress parameters of non-etiolated (Non-Et), etiolated (Et) and de-etiolated (De-Et) maize seedlings with or without the foliar spray of water and PGRs. **, $P<0.01$. Means sharing the same alphabet differ non-significantly ($P>0.05$)

indicated severely reduced (by ~60%) AsA contents. All foliar spray treatment on de-etiolated seedlings led to regain of the AsA contents but the KIN (24% regain) was highly promising (Table 2). The NIA contents were also increased with foliar spray treatments while AsA indicated the greatest increase (10%) in non-etiolated seedlings. Etiolated seedlings exhibited nearly 50% decline in the NIA

Table 2: Accumulation of phenolic compounds and some vitamins in control, etiolated and de-etiolated maize seedlings with or without the foliar spray of water and PGRs

Etiolation (Et) treatments	Foliar spray (FS)	Soluble phenolics (µg/g fresh weight)	Flavonoids (µg/g fresh weight)	Anthocyanins (A_{535})	Ascorbic acid (µmol/g fresh weight)	Niacin (µmol/g fresh weight)	Riboflavin (µmol/g fresh weight)
Non-etiolated	No spray	30.30±1.83de	7.39±0.45	0.350±0.015b	6.24±0.51	0.64±0.04	5.04±0.30
	Water	31.62±1.83cd	8.07±0.58	0.358±0.018b	6.65±0.45	0.68±0.04	5.41±0.38
	AsA	35.05±1.96b	8.32±0.47	0.406±0.025a	6.94±0.42	0.69±0.04	5.61±0.31
	TU	33.85±2.26bc	8.12±0.54	0.397±0.012a	6.78±0.45	0.68±0.05	5.56±0.36
	CCC	38.49±1.46a	8.81±0.55	0.410±0.031b	6.49±0.44	0.68±0.05	5.76±0.36
	Kin	35.55±1.60ab	8.23±0.51	0.397±0.017b	6.79±0.49	0.68±0.05	5.80±0.50
No-et→Et	No spray	17.66±1.92g	4.38±0.38	0.192±0.006f	2.43±0.13	0.31±0.01	2.83±0.31
	Water	17.65±1.00g	4.81±0.28	0.193±0.013f	2.75±0.09	0.35±0.01	3.03±0.32
	AsA	22.20±2.04f	5.45±0.49	0.239±0.010d	2.81±0.16	0.38±0.02	3.39±0.33
	TU	21.87±2.05f	5.35±0.29	0.224±0.011de	2.71±0.17	0.36±0.02	3.39±0.33
	CCC	19.37±2.02g	5.67±0.40	0.206±0.015ef	2.64±0.16	0.36±0.02	3.46±0.27
	Kin	21.23±2.65f	5.75±0.33	0.231±0.009de	2.75±0.15	0.39±0.02	3.77±0.28
Et→Det	No spray	27.17±1.54e	6.83±0.38	0.268±0.012c	4.50±0.30	0.44±0.03	4.65±0.25
	Water	27.37±1.26e	7.29±0.62	0.278±0.010c	4.81±0.31	0.55±0.03	4.86±0.41
	AsA	35.62±2.19ab	8.17±0.40	0.360±0.015b	5.42±0.33	0.57±0.03	5.26±0.26
	TU	35.39±1.21ab	8.18±0.40	0.356±0.017b	5.38±0.29	0.59±0.03	5.17±0.26
	CCC	35.97±1.72ab	8.23±0.37	0.366±0.016b	4.82±0.29	0.58±0.03	5.49±0.25
	Kin	34.80±2.59b	8.35±0.62	0.351±0.019b	5.56±0.32	0.59±0.03	5.56±0.41
	SE (Et × FS)	1.545*	ns	0.013**	ns	ns	ns

*, $P < 0.05$; **, $P < 0.01$; ns, $P > 0.05$. Means sharing same alphabet differ non-significantly ($P > 0.05$)

contents despite foliar spray of PGRs. However, in de-etiolated seedlings, NIA contents improved with all PGRs and the greatest increase (~34%) was noted with TU spray (Table 2). In non-etiolated seedlings RIB contents increased with PGRs spray but the highest increase (15%) was noticed with foliar spray of KIN. In etiolated seedlings reduced RIB decreased despite foliar spray of PGRs, but KIN spray led to a lowest decline (33%). De-etiolated seedlings exhibited a regain in NIA content with PGRs spray while KIN (with 20% regain) was highly effective (Table 2).

Trendlines and correlations

Trend-lines were set and correlations were drawn of the seedling dry weight with the physiological attributes under all three condition based on the PGRs foliar spray (Fig. 4). In non-etiolated seedlings, *Pn*, PHE, FLA, ANT, NIA and RIB were positively correlated while H_2O_2 and MDA were negatively correlated. In etiolated seedlings, none of the physiological attributes was significantly correlated with seedling dry weight. For de-etiolation seedlings, all the physiological characters were positively correlated except *Ci*, H_2O_2 and MDA which were negatively correlated. However, AsA was not correlated with shoot dry weight (Fig. 4).

Discussion

It is well established that light triggers almost all the plant phenomena after either its absorption or by acting as a signal (Lau and Deng, 2010). However, the clear evidence on the role of light in modulating the PGRs action is not yet available. In this study etiolation for six days resulted not only in the substantial elongation of seedlings but also led to loss of seedling dry mass, eventually giving a high shoot elongation/root dry weight ratio. Compared to unsprayed or water-sprayed seedlings the foliar spray of PGRs partially nullified the influence of etiolation, which indicated the effectiveness of PGRs in a regain of dry weight and a decline in the elongation of seedlings. Although all the PGRs were effective in producing these changes, KIN and CCC were quite more effective than the others (Fig. 1). It is reported that light integrates the phytohormonal signaling by phytochrome interacting factors (PIFs) in incorporating the morphogenetic changes (Lau and Deng 2010). These data suggested that although light has its own basic role in modulating the plant growth (Hossain and Kamaluddin 2011; Liu *et al.* 2017), it appeared to synergistically improve the plant efficiency with the foliar spray of PGRs in non-etiolated and de-etiolated seedlings.

Photosynthesis comprises two main of reactions; light reactions (to harness solar energy with photosynthetic pigments and generating reducing powers) and dark reactions (for the production of assimilates using CO_2, water and reducing powers). In this study we found that all the PGRs enhanced the biosynthesis of *Chl* and *Car* but the effects of PGRs were well pronounced on de-etiolated seedlings. Among the PGRs foliar applied in this study, KIN and CCC were more effective than TU and AsA in improving pigment contents (Fig. 2) and leaf gas exchange (Table 1). For plants KIN is a natural growth promoter (Klíčová *et al.* 2004; Petrasek *et al.* 2019) while CCC is a synthetic growth retardant (Kumar and Sharma 2019; North *et al.* 2010). During the biosynthesis of *Chl*, the synthesis of 5-aminoleuvolinic acid (5-ALA), a committed step in the *Chl* biosynthesis (Eckhardt *et al.* 2004; Steccanella *et al.* 2015), is either independent of light (Meller and Grassman 1982) or its synthesis is stimulated under low light intensity and inhibited under high light intensity (Aarti *et al.* 2007). The cytokinins (including KIN) enhance the biosynthesis of

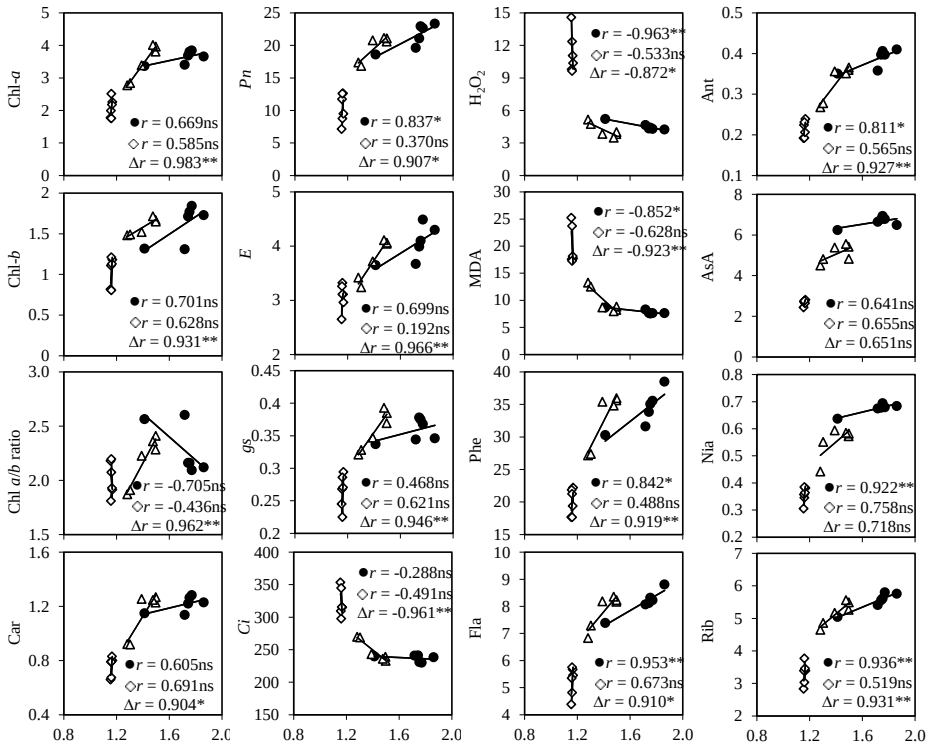

Fig. 4: Trendlines and Pearson's correlation coefficients (*r*) of shoot dry weight with physiological variables of control (non-etiolated; ●), etiolated (◇) and de-etiolated (Δ) seedlings of maize under foliar spray of plant growth regulators (n=6)

chlorophyll by promoting the synthesis of its precursor (5-ALA) when applied exogenously (Jamei *et al.* 2008). Likewise, PGRs including CCC, TU and AsA are also reported to improve the leaf *Chl* and *Car* contents (Borraccino *et al.* 1994; Wahid *et al.* 2017; Arshad *et al.* 2019), but likely mechanisms of action of these PGRs in either improving the synthesis or curtailing the loss of chlorophylls in etiolated and de-etiolated plants are obscure yet. In this study, the etiolated seedlings measured for the photosynthetic pigment contents revealed that less of *Chl a* and more of *Chl b* contents were reduced resulting in an enhanced *Chl a*/*b* ratio, while *Car* was severely declined in unsprayed etiolated maize seedlings. The foliar spray of PGRs was poorly effective in improving photosynthetic pigment decline during etiolation. However, during de-etiolation there was a swift *de novo* synthesis of these pigments, reaching about the level of non-etiolated plants in six days especially with the foliar of KIN and CCC (Fig. 2). The presence of strong positive correlations in the photosynthetic pigment contents and seedling dry weight of de-etiolated seedlings validated our standpoint (Fig. 4). Although this study has clearly revealed the positive effects of foliar spray of the PGRs, further studies are mandatory to elucidate the mechanisms of these PGRs in improving the photosynthetic pigment contents during etiolation/de-etiolation swapping.

In this research, gas exchange properties of leaves indicated that *Pn*, *E*, and *gs* were severely reduced due to etiolation, while *Ci* was increased (Table 1). Shifting of the plants to light chamber for de-etiolation quickly improvised these attributes, while *Ci* was reduced (Fig. 2). In etiolated seedlings, hampered leaf gas exchange is likely due to depletion of the levels of reducing powers with loss of photosystems (*PSI* and *PSII*) in etiolated seedlings, which showed a quick regain (especially of *PSII*) upon greening (Baker and Butler 1976). This revealed that during etiolation the plants suffered great limitation in the availability and utilization of resources to carry out leaf gas exchange in the production of photoassimilates, while the foliar spray of different PGRs was partially helpful in the sustained photosynthetic pigment content and leaf gas exchange. These changes were similar to those observed in the abiotically stressed plants (Cassola *et al.* 2019), hence supporting the view that etiolation is also a stress factor. The positive correlations of seedling dry weight with the *Pn*, *E* and *gs* while negative with *Ci* in de-etiolated seedlings but not in non-etiolated and etiolated seedlings (Fig. 4) witnessed that upon exposure to light the effectiveness of the PGRs was evident from a gain in dry mass (Fig. 1) upon being relieved from light limited condition.

To substantiate further that whether or not absence or low-availability of light produces the effects like other abiotic stresses in the etiolated seedlings, the measurements

were made for changes in the level of H_2O_2 (a representative ROS) and MDA (a membrane lipid peroxidation product) to determine the extent of oxidative damage in non-etiolatied, etiolated and de-etiolated seedlings. The results revealed no great changes in both H_2O_2 and MDA content of non-etiolated plants with or without foliar spray treatments. However, when the seedlings were shifted to darkness for etiolation, non-sprayed or water sprayed seedlings exhibited highest accumulation of both H_2O_2 and MDA, while PGR's sprayed seedlings manifested quite lesser (10 to 15%) decline in the amounts of both these metabolites. However, de-etiolated plants revealed a substantial reduction (more or less 100%) in the level of both H_2O_2 and MDA, which was similar to or lesser than the level measured in non-etiolated plants (Fig. 3). The accumulation of both these metabolites in etiolated maize seedlings was similar to that accumulated in the abiotically stressed plants (Turan and Tripathy 2013; Hameed *et al.* 2014). Although foliar spray of PGRs was lowly effective in reducing the concentration of H_2O_2 and MDA, we believe that etiolation causes oxidative damage and weak cell walls and elongated internodes phenotypes may be the negative consequence of this phenomenon (Sinclair *et al.* 2017). Although no reports are available on the effects of ROS on the cell wall properties in plants, it is reported that high ROS levels were responsible for damage to the cell wall of *Candida albicus* and *Aspergillus niger* (Athie-García *et al.* 2018). The diminution of both these metabolites in de-etiolated seedlings (exhibiting reversal to normal growth) and their tight inverse correlations with dry weight of de-etiolated seedlings (Fig. 4) further supported our notion. Nevertheless, this aspect needs to be investigated comprehensively in plants.

To substantiate that the antioxidative defense is modulated in maize seedlings during etiolation/de-etiolation switch over in this study, the levels of phenolics (PHE, FLA, ANT) and vitamins (AsA, NIA, RIB) were measured (Table 2). It is established that antioxidants are synthesized in abiotically stressed plants to scavenge the ROS and improving the functional properties of cellular membranes (Zhishen *et al.* 1999; Jaleel *et al.* 2009; Derakhshani *et al.* 2017; Derakhshani *et al.* 2017). Our data showed that levels of the measured phenolics and vitamins remained fairly constant in non-etiolated seedlings; declined markedly in etiolated seedlings, but showed a regain in de-etiolated seedlings (Table 2). Taken together, changes in the oxidative stress and antioxidants accumulation, it was found that all these changes were consistent but contrary to each other (Fig. 3). This indicated that etiolation induced the oxidative damage, and reduced the ability of maize to synthesize antioxidants (phenolics and vitamins). However, lack of association of seedling dry weight with phenolics and vitamins in etiolated seedlings and strong positive association of these attributes in de-etiolated seedlings (Fig. 4), revealed the interactive role of light and the PGRs in the synthesis of antioxidants (phenolics and vitamins) and seedling development during de-etiolation. This clearly supports our view that light is pivotal in regulating the plant phenomena with the foliar spray of the PGRs.

Conclusion

The hypothesis that light clearly augments the PGRs action in maize seedlings during de-etiolation was accepted based on the improvements occurring in the leaf pigment contents and gas exchange properties, reduced oxidative damage and improved phenolics and vitamin contents. These findings were further strengthened with the establishment of significant correlation between shoot dry weight and metabolic attributes of maize seedlings, which were significant in the de-etiolated seedlings due to the foliar spray of PGRs. Among the PGRs, the KIN was the most effective followed by CCC, TU and AsA. The molecular mechanism of action of these PGRs during etiolation/de-etiolation transitions warrants further studied.

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