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EXOGENOUSLY APPLIED 5-AMINOLEVULINIC ACID MEDIATED PHYSIOCHEMICAL REGULATIONS AMELIORATE WEAK LIGHT STRESS IN TOBACCO SEEDLINGS

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Abstract

Experiments were conducted to study the deleterious impacts of low intensity light on physiochemical and agronomic attributes of tobacco, to evaluate varying doses of foliar 5-aminolevulinic acid (5-ALA) for alleviation of adverse impacts of low light intensity and to observe either 5-ALA modulated physiochemical regulations impart stress tolerance at agronomic level. Significant decrease of biomass accumulation, synthesis of osmo-protectants, chlorophyll contents, and chlorophyll fluorescence and increase in malondialdehyde were recorded compared to control. Exogenous application of 5-ALA excellently alleviated adverse impacts of low light intensity stress on agronomic and physiochemical attributes of tobacco seedlings. Conclusively, Light stress had adverse implications on all studied attributes while 5-ALA at 10-20 mg/l had remarkable alleviated deleterious impacts of light stress on plant.

Introduction

The productivity of crop is lagging far behind compared to its achievable potential because of low temperature and sunlight intensity at earlier stages of growth in Southwest China (Jiang *et al.* 2014). Coincidence of colder temperature and poor light intensity with early stages of development often leads to poor development of seedlings and ultimately deteriorates quality of tobacco leaves (Jin *et al*. 2014).

Low intensity of light hampers the electron flow in photosystem and thereby impair the biosynthesis of reductants at the end of light reactions (Yang *et al*. 2018). Consequences of poor efficacy of light reactions are limited availability of reductants and poor energy yields of light reactions (Li *et al.* 2018). Whereas, carbohydrates are prerequisites for growth slow down the growth rate and biomass accumulation (Townsend *et al.* 2018). Likewise, decrease in fluorescence of chlorophyll also decreases energy output of light reactions and eventually affect growth (Gao *et al*. 2018).

Stress conditions can be alleviated using numerous agronomic stratagems like plant growth substances. Among them, 5- aminolaevulinic acid (5-ALA) is a potent regulator of growth, enzymes, precursor in biosynthesis of chlorophyll and numerous physio-morphological attributes (Niu and Ma 2018). Exogenously applied 5-ALA enhances the synthesis of antioxidants, chlorophyll, soluble proteins and photosynthetic efficacy which impart stress tolerance (Farid *et al*. 2018).

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Information about the adverse impacts of low light intensity to tobacco is scarce in previous studies. While, little is known about the 5-ALA modulated physiochemical regulations in tobacco since most of aforementioned studies have been conducted on other crops. Hence the present study was conducted to investigate adverse impacts of low light intensity in tobacco using physiochemical and morphological attributes as indicators, to optimize the exogenous 5-ALA as potent alleviator of adverse implications of low light on tobacco and to explore either 5-ALA mediated physiochemical transformations induce stress tolerance at morphological level.

Materials and Methods

The experiment was conducted at College of Agronomy and Biotechnology, Southwest University, Chongqing (29°49'32" N, 106°26'02" E; 220 m a.s.l.), China, from 2 June to 25 July 2016. Tobacco (*Nicotiana tabacum* K326) seedlings were grown in Petri plates, and treatments were applied after 40 days when the seedlings were with 5 functional leaves. The experiment was carried out in the greenhouse incubator (12 hrs light/25°C; dark 12 hrs/16 °C; intensity of illumination 200 μ mol m^{-2} s^{-1}; low light stress was the intensity of illumination l00 μ mol m^{-2} s^{-1}.). 5-ALA was foliar applied at 5 different concentrations. Treatments were comprised of A = intensity of illumination 200 μ mol $m^{-2}s^{-1}$ + distilled water (control); A0 = Light stress + distilled water; A5 = Light stress + 5 mg/l 5-ALA; A10 = Light stress + 10 mg/l 5-ALA; A20 = Light stress + 20 mg/l 5-ALA; A40 = Light stress + 40 mg/l 5-ALA and A80 = Light stress + 80 mg/l 5-ALA. Whereas, foliar treatments were carried out on plants, second and third spray was applied again after 3 days interval to exploit full potential of application.

The experiment was conducted in CRD and replicated 3 times. Data were analyzed by ANOVA using the SPSS software (SPSS, version 25.0; IBM Corporation, Armonk, New York, USA) (Steel *et al*. 1997).

After measuring the height and fresh weight the seedling was placed in oven at 105°C for 20 min to stop respiration, followed by drying at 70°C for 48 hrs to determine the dry weight. The malondialdehyde (MDA) content was assayed through thiobarbituric acid (TBA) assay (De-Vos *et al*. 1991). Free proline content was measured using the ninhydrin method (Bates *et al*. 1973). Soluble protein content was determined using coomassie brilliant blue method (Bradford 1976). Determination of soluble sugars was done by anthrone color method (Zhu *et al*. 2012). Chlorophyll and carotenoid content were measured using the method of Wellburn (1994). Fluorescence parameters were measured using a PAM-2000 portable pulsed modulation fluorometer (Quick and Stitt 1989, Van and Snel 1990).

Results and Discussion

Relatively lesser plant height was recorded under A80 (3.50 cm) compared to other treatments. While, higher and statistically similar plant height was observed in other treatments. Statistically similar and more stem diameter and dry weight were measured under A and A10 compared to other treatments. Whereas, statistically alike and higher fresh weight and root/shoot were recorded under A, A10 and A20 compared to other treatments. Contrarily, relatively lesser stem diameter, fresh and dry weight and root/shoot were recorded under A80 compared to other treatments (Table 1).

Statistically similar and significantly lesser synthesis of MDA was quantified under A10, A20, A40 and A80 compared to other treatments. While, statistically alike and more proline was recorded under A, A0 and A5 compared to other treatments. Whereas, similar and more soluble proteins were observed under A, A5, A10, A20 and A40 compared to other treatments. Lesser

synthesis of soluble sugars was observed under A0 while statistically similar and higher soluble sugars were quantified under all other treatments (Table 2).

Table 1. Effect of foliar 5-aminolevulinic acid on biomass accumulation of tobacco seedlings under low light intensity stress.

Treatments	Plant height (cm)	Stem diam (mm)	Fresh weight (g/plant)	Dry weight (g/plant)	Dry root/shoot ratio
A	3.80 ± 0.27ab	3.69 ± 0.22a	2.92 ± 0.20a	0.14 ± 0.01a	0.12 ± 0.01a
A0	3.94 ± 1.01ab	3.38 ± 0.19ab	2.16 ± 0.48bc	0.09 ± 0.02b	0.08 ± 0.00bc
A5	3.77 ± 0.32ab	3.12 ± 0.08b	2.03 ± 0.04c	0.09 ± 0.00b	0.08 ± 0.01c
A10	4.72 ± 0.39a	3.72 ± 0.20a	2.87 ± 0.33ab	0.13 ± 0.01a	0.11 ± 0.02ab
A20	4.13 ± 0.71ab	3.20 ± 0.38b	2.22 ± 0.70abc	0.10 ± 0.03b	0.10 ± 0.02abc
A40	3.71 ± 0.84ab	3.17 ± 0.40b	1.96 ± 0.40c	0.09 ± 0.02b	0.08 ± 0.00c
A80	3.50 ± 0.28b	3.04 ± 0.18b	1.73 ± 0.16c	0.08 ± 0.01b	0.07 ± 0.02c

Values are mean ± SE. Values followed by different letters within each column are significantly different according to DMRT ($p < 0.05$).

Table 2. Effect of foliar 5-aminolevulinic acid on membrane stability and osmo-protectants of tobacco seedlings under low light intensity stress.

Treatments	MDA (nmol/g)	Free proline (µg/g)	Soluble protein (mg/g)	Soluble sugars (mg/g)
A	6.21 ± 2.00a	41.34 ± 14.83a	8.21 ± 1.03a	3.16 ± 0.98ab
A0	9.78 ± 1.65a	31.40 ± 4.80ab	6.33 ± 0.35b	1.89 ± 0.67b
A5	8.53 ± 0.15b	28.53 ± 5.06ab	6.98 ± 0.48ab	2.38 ± 0.94ab
A10	7.38 ± 2.24c	25.41 ± 6.30b	6.77 ± 0.27ab	2.68 ± 0.88ab
A20	7.03 ± 1.59c	11.31 ± 9.06c	7.04 ± 1.49ab	4.11 ± 1.43a
A40	7.85 ± 0.81c	7.66 ± 3.77d	7.10 ± 1.22ab	2.68 ± 0.95ab
A80	7.76 ± 1.21c	19.64 ± 5.11bc	2.10 ± 0.53c	2.39 ± 0.31ab

Statistically similar and more chlorophyll *a* was recorded under A, A10, A40 and A80 compared to other treatments. Whereas, statistically alike and higher biosynthesis of chlorophyll *b* and carotenoids were observed under A0, A40 and A80. While, almost similar and higher chlorophyll *a/b* were quantified under other treatments compared to A0 (Table 3).

Statistically alike and more minimum fluorescence (F0) was observed under A, A5, A10 and A80 compared to other treatments. While, significantly higher maximum fluorescence (Fm), minimum fluorescence after 5 min (F0') and maximum fluorescence after 5 min (Fm') were recorded under A compared to other treatments. Likewise, statistically similar and more photochemical efficiency (Fv/Fm) was quantified under A, A40 and A80 compared to other treatments (Table 4).

Improvement of biomass accumulation under light stress and exogenous 5-ALA can be attributed to its role in biosynthesis of chlorophyll. Improvement of chlorophyll contents might have enhanced the synthesis of carbohydrates and more partitioning of carbohydrates towards

green parts resulted in accumulation of biomass. However, biomass accumulation did not enhance linearly with increasing concentrations of exogenous 5-ALA. It can be ascribed to inhibitory effect at higher concentrations. Moreover, poor accumulation of biomass at higher concentrations of 5-ALA can be defined in context of higher MDA and lesser synthesis of osmo-protectants. Light stress might have slowed down the synthesis of osmo-protectants which ultimately aggravated oxidative stress and therefore slowed the accumulation of biomass. Application of 5-ALA enhanced the synthesis of osmo-protectants and biomass accumulation under stress conditions (Xiong *et al*. 2018).

Table 3. Effect of foliar 5-aminolevulinic acid on stay green trait of tobacco seedlings under low light intensity stress.

Treatments	Chl *a* (mg/g)	Chl *b* (mg/g)	Carotenoid (mg/g)	Total chlorophyll (mg/g)	Chl *a*/*b*
A	1.05 ± 0.30ab	0.41 ± 0.11b	0.14 ± 0.04d	1.56 ± 0.42ab	2.56 ± 0.07ab
A0	0.94 ± 0.14b	0.49 ± 0.06ab	0.23 ± 0.03a	1.43 ± 0.21ab	1.91± 0.01b
A5	0.99 ± 0.06b	0.38 ± 0.02b	0.17 ± 0.01cd	1.37 ± 0.08b	2.61± 0.04a
A10	1.04 ± 0.11ab	0.41 ± 0.04b	0.18 ± 0.03bc	1.45 ± 0.16ab	2.54 ± 0.02ab
A20	1.06 ± 0.03b	0.38 ± 0.02b	0.18 ± 0.01bc	1.44 ± 0.05b	2.79± 0.04a
A40	1.32 ± 0.07a	0.53 ± 0.04a	0.23 ± 0.02a	1.85 ± 0.12a	2.49 ± 0.03ab
A80	1.22 ± 0.19a	0.49 ± 0.09ab	0.22 ± 0.02ab	1.71 ± 0.31ab	2.49 ± 0.10ab

Table 4. Effect of foliar 5-aminolevulinic acid on chlorophyll fluorescence of tobacco seedlings under low light intensity stress.

Treatments	F0	Fm	Fv/Fm	F0'	Fm'
A	377.50 ± 27.58ab	2098.00 ± 19.80a	0.82 ± 0.01a	340.00 ± 21.21a	1301.50 ± 13.44a
A0	363.50 ± 26.16b	1497.00 ± 118.79c	0.76 ± 0.00bc	276.00 ± 8.49bc	651.50 ± 13.44bc
A5	425.00 ± 25.46a	1498.50 ± 178.90c	0.71 ± 0.05c	309.50 ± 19.09ab	645.50 ± 9.19bc
A10	387.00 ± 1.41ab	1594.50 ± 86.97bc	0.76 ± 0.01bc	280.00 ± 8.49bc	622.00 ± 60.81bc
A20	358.00 ± 0.00b	1532.00 ± 22.63c	0.77 ± 0.00b	259.00 ± 9.90c	584.00 ± 55.15c
A40	344.50 ± 19.09b	1549.00 ± 62.23bc	0.78 ± 0.00ab	260.50 ± 24.75c	631.50 ± 92.63bc
A80	390.00 ± 12.73ab	1765.00 ± 29.70b	0.78 ± 0.00ab	298.50 ± 16.26bc	739.00 ± 62.23b

F0 = Minimum fluorescence; Fm = Maximum fluorescence; Fv/Fm = Photochemical efficiency;
F0' = minimum fluorescence after 5 min; Fm' = Maximum fluorescence after 5 min.

Light stress might promote the synthesis of reactive oxygen species which ultimately triggered lipid peroxidation of membranes. Hence, capability of cells to retain water might have decreased and consequently growth was slowed down. Moreover, light stress might have triggered the breakdown of chlorophyll. Accelerated breakdown of chlorophyll might decrease the availability of carbon skeleton which is prerequisite for synthesis of proline, soluble proteins and soluble sugars (Shahid *et al*. 2017). Moreover, stress triggered disruptions in physiochemical attributes decided strong correlation with morphological attributes and thus negative impacts were recorded at agronomic level also (Saleem *et al*. 2017).

Application of 5-ALA enhanced net photosynthetic rate, photochemical quenching and photosynthetic activity of leaf based on the electron transport rate of photosystem-II, ultimately tolerance against stress conditions was improved (Niu and Ma 2018). Likewise, foliar applied 5-ALA enhanced activities of antioxidants, synthesis of chlorophyll and decreased leakage of electrolytes under stress conditions (Farid *et al.* 2018).

Improvements in biosynthesis of chlorophyll, fluorescence and quantum yield of photosynthesis can be attributed to 5-ALA mediated boost in activities of protochlorophyllide. Upregulations in synthesis of protochlorophyllide might have acted as substrate for synthesis of chlorophyll and consequence in improvements of chlorophyll contents. Foliar application of 5-ALA inhibited the synthesis of aminolaevulinic acid dehydratase and porphobilinogen deaminase which decreased the degradation of chlorophyll (Hemantaranjan *et al.* 2014). Likewise, improvements in fluorescence of chlorophyll and quantum yield of photosynthesis under availability of 5-ALA can also be attributed to antioxidative and protective action of proline, soluble sugars and proteins. Accumulation of osmo-protectants under availability of 5-ALA might have enhanced the solute concentration and thus reduced the sensitivity towards light intensity. Exogenous application of 5-ALA under stress conditions improved accumulation of osmo-protectants, net photosynthetic rate and stomatal conductance (Kosar *et al.* 2014). While application of 5-ALA enhanced the biosynthesis of chlorophyll under stress environments (Feng *et al.* 2015). Likewise, availability of 5-ALA under stress improved carbon fixation, decreased photoinhibition and osmotic stress (Niu and Ma 2018).

The results of the present study revealed that imposition of light stress deleteriously impacted biomass accumulation, membrane stability, synthesis of osmo-protectants, chlorophyll contents, and chlorophyll fluorescence in tobacco seedling, while exogenously applied 5-ALA effectively alleviated adverse implications of light stress on these attributes. More remarkable agronomic, biochemical and physiochemical responses were recorded with 10 - 20 mg/l exogenous 5-ALA under light stress.

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