Effects of LED light spectra on active oxygen metabolism and expression of antioxidant isozymes in *Houttuynia cordata* Thunb. seedlings

ZhaoQing Wang [1], JingYun Tian [2], Liyan Yang [3*]

[1, 2, 3]Shanxi Normal University, Linfen, 041004, P.R.China

Abstract— *The effects of various LEDs on active oxygen metabolism and patterns of SOD, POD and CAT isozymes in Houttuynia cordata Thunb. seedlings were investigated. After three weeks of light treatment, the MDA content was higher under blue LED compared with the control ($P < 0.05$), while it decreased under white, red and yellow LEDs ($P < 0.05$). The content of H_2O_2 was gradually increased in red, yellow, green and blue LEDs. The production rate of superoxide anion increased under yellow and blue LEDs by contrast with the control ($P < 0.05$), and it decreased under white LED ($P < 0.05$). LEDs altered the banding patterns of POD enzymes where the more loci of POD isozymes were observed under green and blue LEDs. The increased intensities of Fe-SOD were showed in green and blue LEDs. As for Mn-SOD and CAT enzymes, enhanced intensities appeared in all LED groups compared with the control. Our results indicated that the antioxidant system of Houttuynia cordata seedlings were more sensitive to short light wavelength than the long ones.*

Keywords—Houttuynia cordata* Thunb*, LED, light spectrum, Oxidative stress

I. INTRODUCTION

Among environment factors, light is not only an essential energy source for plant but also an important signal influence plant development, biosynthesis and gene expression throughout the life cycle of a plant [1-2]. The light spectrum affects growth and photosynthesis of plants has been well demonstrated in many species [3-4].

The light-emitting diodes (LED) is a unique type of semiconductor diode which showed great advantages over existing indoor agricultural lighting wherein the adjustable light intensity and quality, high photoelectric conversion efficiency, longer life, and low thermal output, and so on [5]. LED has been applied in plant disease prevention [6], plant tissue culture [7-8], space agriculture [9] and alga incubation [10-11]. Some studies demonstrated that LED with certain spectra may improve antioxidant activity of pea seedlings [12]; influence antioxidant properties as well as growth and photosynthesis of leaf lettuce [13-14].

Houttuynia cordata Thunb. is one of authorized medical and edible plants by China's Ministry of Health. It is rich in nutritious for human being including certain secondary metabolites as rutin, quercitrin and quercetin which showed antioxidant effects. Li concluded light intensity affected yield and quality of *Houttuynia cordata* Thunb.[15],while there is little knowledge about oxidative metabolism in *Houttuynia cordata* Thunb. under different LED light spectra. In the present study, we investigated different LED light spectra on the contents of malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and reactive oxygen species (ROS) of *Houttuynia cordata* Thunb. We also analyzed the alteration of antioxidant isozyme patterns.

II. MATERIALS AND METHODS

2.1 Plant material and light treatments

The rhizoma of *Houttuynia cordata* Thunb. were from Yichang city, Hubei province, China, and were cultivated in plastic containers with nutrient solution in a controlled growth chamber at 25 ºC /20 ºC (day/night), with a 12 hr photoperiod per day, 75% relative humidity and a photosynthetic photon flux density of 40 umol / (m^2.s). When the third leave fully expanded, seedlings were treated with various LED lights. Six treatments were designed as following: white LED (W), red LED (wavelength 620-630nm, R), yellow LED (wavelength 585-590nm, Y), green LED (wavelength 515-520nm, G) and blue LED (wavelength 455-460nm, B), the fluorescent lamps were used as the control (CK). Three weeks later, the seedlings were ready for analysis.

2.2 Measurements

2.2.1 The contents of MDA and H_2O_2

MDA content was measured according Ye and Zhu [16] with slight modifications. The supernatant of material grinding solution was precipitated with 0.6% (w/v) 2-thiobarbituric acid. The reaction mixture was incubated in a water-bath shaker at 100 ºC for 30 min. The amount of MDA was estimated as micromole per gram FW.

H_2O_2 extracttion was made from 0.5 g of the treated plant in ice-cold acetone. By addition of 5% (w/v) titanic sulfate and ammonia, the peroxide–titanium complex was precipitated then dissolved H_2SO_4, the absorbance was read at 412 nm. H_2O_2 content was calculated from a standard curve prepared in similar way [17].

2.2.2 The generation rate of superoxide anion

The production rate of superoxide anion was determined by the hydroxylamine method [18]. Briefly, fresh leaf sample was homogenized in phosphate buffer (65mmol/L, pH7.8), then centrifuged. The supernatant was mixed with hydroxylamine hydrochloride and kept at 25°C for 20min. α-Naphthylamine and aminobenzenesulfonic acid were used as chromogenic agents and kept at 25°C for 20min. Absorbance of the supernatant was measured at 530 nm by spectrophotometer (722S, Vis spectrophotometer, JingHua).

2.2.3 The electrophoresis of antioxidant enzymes

To determine the variation in the expression pattern of antioxidant enzyme， the leaves (0.2 g) from different treatments were grinded into homogenate with phosphate buffer (50mM, pH 7.8), containing 1% PVP. The grinding fluid was centrifuged and the supernatant was collected as crude enzyme.

Superoxide dismutase (SOD) isozymes were dyed as colorless bands on the gels, incubated in NBT under dark condition. The gel was immersed in phosphate buffer (0.05M, pH 7.8), and the different isozymes of SOD were identified by staining the gels again after previous incubation at 25 ºC for 30 min in 5 mM KCN or 5 mM H_2O_2. Cu/Zn-SOD were inhibited by KCN, Fe-SOD were inactivated by H_2O_2, while Mn-SOD are resistant to both the inhibitors [19-21].

Peroxidase (POD) isozymes were visualized as pale blue bands by incubating the gels for 10-15 min in the dye liquor containing 5M acetic acid, 1.5M sodium acetate, 70 ml of distilled water and 3-5 drops of H_2O_2 [16] .

Isozyme bands for catalase (CAT) were showed by soaking the gel in 0.03% H_2O_2 for 10 min under darkness and oscillation. After rinsed three times with distilled water, the gel was stained in a reaction mixture containing 2% (w/v) potassium ferricyanide and 2% (w/v) ferric chloride (1:7). The isozymes appeared as bright yellow bands on a brown background [22].

2.3 Statistical analysis

All the data give as means ± SD (n=3). Statistical significance was estimated at $P<0.05$ according to Duncan's multiple range test and one-way ANOVA with SPSS software program 17.0.

III. RESULTS

3.1 Effects of various LEDs on MDA content

Reactive oxygen species degraded polyunsaturated lipids, forming malondialdehyde [23]. MDA content was significantly increased in blue LED ($P< 0.05$) compared with the rest LEDs, and no statistical differences showed between green LED and the control. They were remarkable decreased under white, red and yellow LEDs compared with the control ($P< 0.05$).

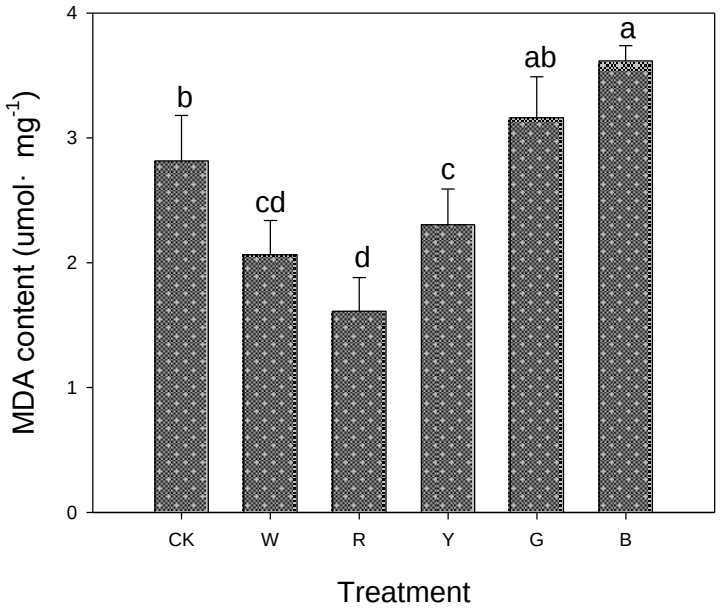

FIG.1 EFFECTS OF DIFFERENT LIGHT SPECTRA ON MDA CONTENT OF *HOUTTUYNIA CORDATA* THUNB. SEEDLINGS.

The means and standard deviations were based on triplicate incubations. Horizontal bars at different levels above the columns indicate significant ($P< 0.05$) differences among the treatments. The same below.

3.2 Effects of various LEDs on ROS level

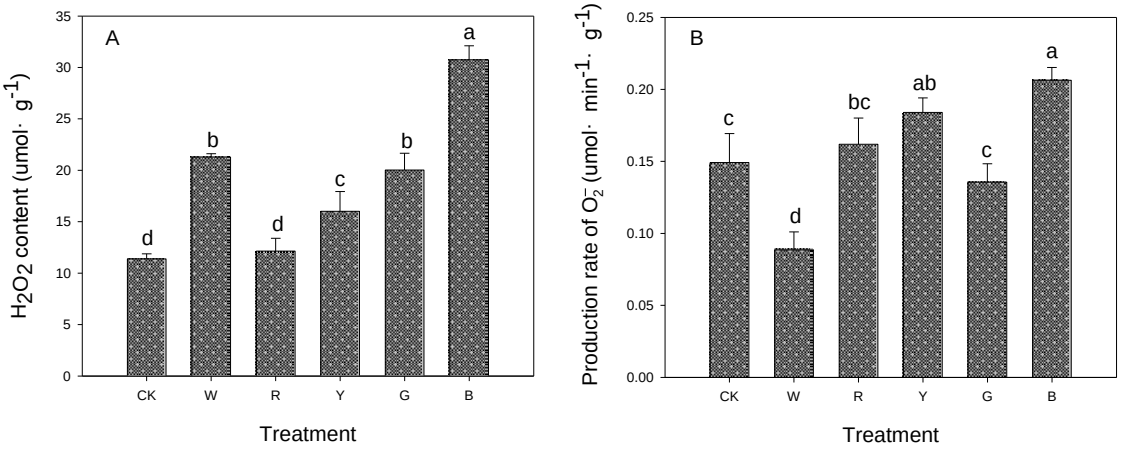

FIG.2 EFFECTS OF DIFFERENT LIGHT SPECTRA ON H2O2 CONTENT (A) AND SUPEROXIDE ANION PRODUCTION RATE (B) OF HOUTTUYNIA CORDATA THUNB. SEEDLINGS.

The H_2O_2 content and the superoxide anion production rate were measured as the representatives of ROS level of *Houttuynia cordata* Thunb. seedlingsq (Fig.2A, 2B). Strikingly increased of H_2O_2 content was observed when *Houttuynia cordata* Thunb. seedings were treated with white, yellow, green and blue LEDs compared with the control ($P< 0.05$), and they were not significantly different between red LED and the control ($P> 0.05$) (Fig.2A). With the decreased LED light wavelength from red to blue, H_2O_2 content increased significantly ($P< 0.05$). Under yellow and blue LEDs, the superoxide anion production rate was remarkably higher than the control ($P< 0.05$), and slightly up in red LED. However, white and green LEDs induced the decreased superoxide anion production rate.

3.3 Superoxide dismutase isozymes pattern of *Houttuynia cordata* Thunb. seedlings under various LEDs

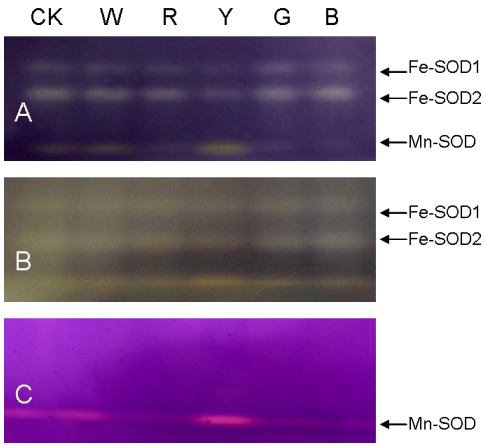

FIG.3 EFFECTS OF DIFFERENT LIGHT SPECTRA ON SOD ISOZYME PROFILE OF HOUTTUYNIA CORDATA THUNB. SEEDLINGS (A: STANDARD DYEING, B: PRE-DYED WITH KCN, C: PRE-DYED WITH H_2O_2)

TABLE 1
THE INTEGRAL OPTICAL DENSITY OF THE ISOZYME OF SOD IN *HOUTTUYNIA CORDATA* TUNB. SEEDLING LEAVES UNDER DIFFERENT LIGHT SPECTRA

Bands of isozyme	Rf	IOD					
		CK	W	R	Y	G	B
Fe-SOD1	0.64	25.85	24.83	36.29	14.35	48.95	55.43
Fe-SOD2	0.70	52.72	43.62	58.06	24.28	72.00	98.80
Mn-SOD	0.83	6.62	10.77	17.58	34.66	15.88	10.07

Plants have evolved an advanced antioxidant enzyme system to eliminate free radicals and ROS during the evolution process. SOD catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. Polyacrylamide gel electrophoresis showed that 3 loci expressed for the SOD isozymes where two loci for Fe-SOD (Fig. 3B) and one for Mn-SOD (Fig. 3C), and Cu/Zn-SOD was not observed in the leaves of *Houttuynia cordata* Tunb. seedlings. The integral optical density (IOD) of Fe-SOD1 and Fe-SOD2 was enhanced under red, green and blue LEDs compared with the control, while it decreased under white and yellow LEDs and the lowest IOD was observed under yellow LED treatment (Table 1). The IOD of both Fe-SOD1 and Fe-SOD2 was higher under green and blue LEDs than that under red and yellow ones. However, it was not the case for Mn-SOD where the higher IOD appeared under red and yellow LEDs than that under green and blue LEDs.

3.4 Peroxidase isozyme pattern of *Houttuynia cordata* Tunb seedlings under different LEDs

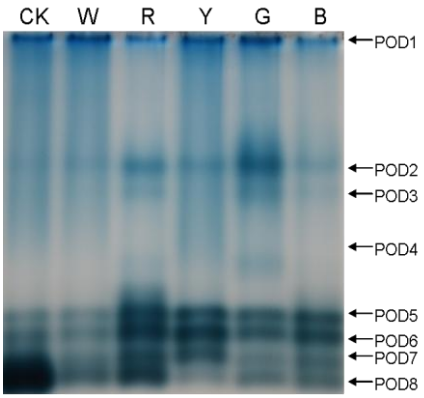

FIG.4 EFFECTS OF DIFFERENT LIGHT SPECTRA ON POD ISOZYME PROFILE OF *HOUTTUYNIA CORDATA* THUNB. SEEDLINGS

TABLE 2
THE INTEGRAL OPTICAL DENSITY OF THE ISOZYME OF POD IN *HOUTTUYNIA CORDATA* TUNB. SEEDLINGS UNDER DIFFERENT LIGHT SPECTRA

Bands of isozyme	Rf	IOD					
		CK	W	R	Y	G	B
POD1	0.17	300.02	419.35	219.59	407.87	370.80	239.77
POD2	0.37	89.15	141.11	251.13	212.63	507.08	146.67
POD3	0.40			114.62	78.41	194.15	6.04
POD4	0.52					111.11	
POD5	0.60	127.43	117.75	382.65	260.79	202.21	264.94
POD6	0.63	160.21	150.57	340.32	343.92	194.20	272.77
POD7	0.67			192.46	321.86	102.07	152.49
POD8	0.70	1442.9	369.61	340.36		163.49	163.27

POD isozymes presented various banding patterns (Fig. 4 and Table 2). The loci that POD expressed were 5, 5, 7, 6, 8 and 7, respectively under the control, white, red, yellow, green and blue LEDs. POD1, POD2, POD5 and POD6 were observed in all treatment groups, while POD4 appeared only in green LED group and POD8 was not observed in yellow LED group. As IOD value showed, LED induced higher intensities of POD2, POD3 and POD7 compared with the rest loci.

3.5 Catalase isozyme pattern of *Houttuynia cordata* Tunb seedlings under different LEDs

FIG. 5 EFFECTS OF DIFFERENT LIGHT SPECTRA ON CAT ISOZYME PROFILE OF *HOUTTUYNIA CORDATA* THUNB. SEEDLINGS

TABLE 3 THE INTEGRAL OPTICAL DENSITY OF THE ISOZYME OF CAT IN *HOUTTUYNIA CORDATA* TUNB. LEAVES UNDER DIFFERENT LIGHT SPECTRA

Bands of isozyme	Rf	IOD					
		CK	W	R	Y	G	B
CAT1	0.48	280.56	683.62	585.98	370.19	1584.05	623.36

One CAT loci expressed under all groups (Fig.5 and Table 3). The IOD values of CAT1 in all the LEDs were higher than the control. The IOD value was the highest in green LED group; it was the lowest under the control.

IV. DISCUSSION

Under stressed environment, ROS levels increased dramatically [24]. Plants have developed various strategies including antioxidant system to alleviate the negative effects of ROS [25]. MDA, as the biomarker of oxidative damage, have applied to determine oxidative injury as a simple and sensitive method [26]. Our results showed higher MDA accumulation under shorter wavelength LEDs as green and blue compared with under longer ones, suggesting the more serious membrane lipids peroxidation under green and blue LED spectra. We believe the higher energy that the shorter wavelength lights own give the reason.

SOD constitutes the first cellular defense line against oxidative stress that eliminate the superoxide anion radical [27]. Our results showed the higher expression of Fe-SOD1 and Fe-SOD2 under green and blue LEDs than under red and yellow ones. However, it is not the case for Mn-SODs. It has been reported that Fe-SODs are abundantly localized in plant chloroplasts

while Mn-SODs were found predominantly in mitochondrion and peroxisomes [28-29]. It turned out that chloroplasts were sensitive to short wavelength light and mitochondrions were more subtle to long wavelengths.

CAT and POD were important H_2O_2 detoxifying enzymes. The present results showed short wavelength induced more POD loci to express, as for CAT, no more loci expressed under shorter wavelength LED, but higher CAT activity presented. All in all, shorter wavelength LEDs lead to more serious lipid oxidation due to the higher energy they exert, thus the more isoenzyme loci expressions of Fe-SOD, POD as well as the higher activity of CAT. Mn-SOD loci expression is exceptional which might due to its localization. This result is in agreement with the level of H_2O_2 content that gradually increased from red to blue LEDs. The present results suggest differential expression of SOD/POD/CAT seem to form important components of antioxidant defense in *Houttuynia cordata* Thunb. leaves under various LEDs which helps to enhance oxidative stress resistance.

V. CONCLUSION

The experiment suggested that the short wavelength light lead to more seriously oxidative damage than longer wavelengths. Upon varied LEDs treatment, short wavelength light promoted the accumulation of ROS which resulted in the improved antioxidase activities and more loci expression. By reason of the different expression of Fe-SOD and Mn-SOD, we deemed that light spectra have unlike mechanism on chloroplasts and mitochondria which need to further investigation.

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