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A case study of photomorphogenic response of AmaranthusviridisL.during de-etiolation of seedlings

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ABSTRACT

This study investigated the photomorphogenic response on A. viridis during de-etiolation of seedlings using LED lights of different wavelength such as blue (450-430 nm), green (560- 520nm)andred(680-640nm)againstwhitelight.Theresultwasanalyzedonthebasisof growth parameters and pigment development. The wavelength of light has a profound effect on de-etiolation of the seedlings. Dark condition enhanced the hypocotyl elongation. Blue, green and white light reversed de-etiolation. Blue light found to has a profound effect onthe lengthofleaflamina.However,redlighthasnoeffectinthereversalofdarkinducedde-etiolation.Biomassproductionwashigherforseedlingsinbluelight forA.viridis.Chlorophyll production was triggered under white light. However chlorophyll synthesis was least responsive to green light and red light in A. viridis. Further studies are required to establish theenzymaticandmolecularmechanismsinvolvedinthechangesofphotomorphogenic responses under various wavelengths of light and dark conditions.

Keywords
Photomorphogenesis,LightEmitting Diodes,De-etiolation

1. Introduction

Light is vital to a plant's growth and survival. Even though plants use the full spectrum of visible light, some wavelengths are more important than others in making photomorphogenic responses. Different wavelengths of light can trigger or inhibit growth and development of plants. Higher plants not only transform solar energy into chemical energy through the process of photosynthesis but also use as an informational cue to control a multitude of physiological responses throughout their life cycle. Collectively these responses are known as photomorphogenesis. (Kami C et al., 2010)

In developmental biology, photomorphogenesis is a light- mediated development, where plant growth patterns respond to the light spectrum. This is a discrete process from photosynthesis where light is used as a source of energy. Perhaps the most important component for encoding the complexity of responses is the multiple families of photoreceptors (Biswas K.K et al., 2003). Seedling responses to light are mediated by at least three classes of regulatory

photoreceptors: (a) phytochromes, which respond mainly to red and far-red light but, which also absorb blue and UV-light; (b) photoreceptorsthatarespecificforblueandUV-Alight;and (c) UV-B photoreceptors. Phytochromes, cryptochromes and phototropins are photochromic sensory receptors that restrict the photomorphogenic effect of light to the UV-A, UV-B, blue and red portions of the electromagnetic spectrum (J JCasal., 2000) (Folta K M et al., 2001). Photosynthetic pigments, for example chlorophylls and carotenoids, have important roles as screening agents for the regulatory photoreceptors. Surprisingly little is known about their direct effects on developmentalresponses.

There are at least three stages of plant development where photomorphogenisis occurs: seed germination, seedling development and the switch from the vegetative to the flowering stage (photoperiodism). In the case of seed germination absence of light, leads to an etiolatedseedling (Josse E et al., 2008). Upon exposure to light, the seedling switches rapidly to photomorphogenesis and the seedlings are said to be de-etiolated (Leivar P et al., 2008) (Von Arnim A et al., 1996).

In the light spectrum, blue light has a wavelength of 450- 430 nm. Green light has a wavelength of 560-520 nm. Red light has a wavelength of 680-640 nm and their confluence make white light. These wavelengths of lights can be provided with the Light Emitting Diodes(LEDs)experimental work in photomorphogenesis (Muneer S et al., 2014) (Kim, H.H et al., 2005). Also, dark condition is used to study the effect of darkness on plant growth and development, skotomorphogensis.

In the present work, the de-etiolation process of A.viridis under different light regimes was studied on the basis of morphological development as well as pigment development.

2. Materials andMethods

2.1 Seedlings underetiolation

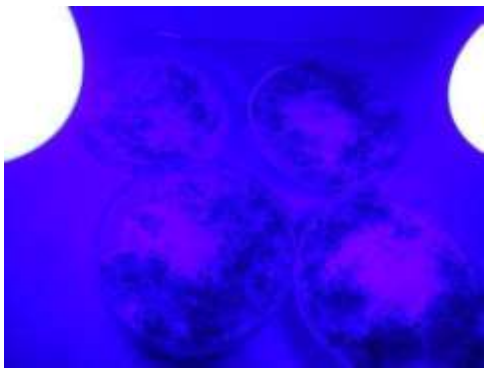
The seeds of A. viridis were collected, washed and sown in petriplates. Water was provided in sufficient quantity for germination. These petriplates were transferred into dark. The seeds germinated in three days.

2.2 Seedlings underde-etiolation

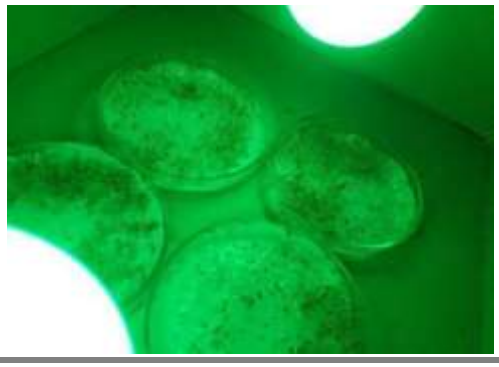
The three days old A.viridis seedlings contained in twelve petriplates were transferred into four different boxes lighted with red, blue, green and white light. Each box was lighted with two LED bulbs (7 watt). Light conditions were provided for 24 hrs/day for 6 days. Also a twelve petriplates consisting of the seedlings were kept under dark for 6days.

[a]

[b]



[c]



[d]

Fig: A. viridis seedlings under de-etiolation. a) white light b) red light c) blue light d) green light

Analysis of the photomorphogenic responses of A. viridis

Three replicates of seedlings were collected at regular intervals and analyzed for growth parameters such as hypocotyl elongation, length of leaf lamina, biomass and also chlorophyllproduction.

a) Hypocotyl elongation and length of leaflamina

The length of hypocotyl and leaf lamina was measured in centimeters using a twine and scale.

b) Quantification ofbiomass

Dry matter content was computed from fresh weight against dry weight. Seedlings were kept for 24 - 48 hours drying oven set at 80℃.

c) Estimation of chlorophyllpigments

Chlorophyll content of seedlings was estimated using Dimethyl Sulfoxide method (Hiscox J.D. et al.,1979). 250 mg of fresh leaf material was suspended in 1 ml of DMSO and incubated it for 20 minute at 60^{o} C in a water bath. The chlorophyll extract was decanted, 1.5 ml of DMSO was added to it and incubated again for 20 minute at 60℃. The final volume of the mixture was made up to 5 ml using DMSO. The amount of chlorophyll was then estimated spectrophotometrically using the followingequations

$$\text{Chlorophyll a } (\mu g/ml) = 12.7(A_{663}) - 2.69(A_{645}) \times V/ (1000 \times W)$$

$$\text{Chlorophyll b } (\mu g/ml) = 22.9 (A_{645}) - 4.68(A_{663}) \times V/(1000 \times W)$$

$$\text{Total chlorophyll}(\mu g/ml) = 20.2(A_{645}) + 8.02(A_{663}) \times V/(1000 \times W)$$

Where, A = absorbance at specific wavelengths, V = final volume of chlorophyll extract in DMSO, W= fresh weight of tissue extracted.

3. Results

Table 1:Hypocotyl length of de-etiolated seedlings of A. viridis

Duration of light (hrs)	Length of hypocotyl (cm)				
	Dark	Red	Blue	Green	White
24	1.56 ±0.59	1.46 ±0.35	0.76±0.05	1.20±0.94	1.34±0.40
48	2.86 ±0.30	3.20 ±0.64	1.26±0.38	2.70±0.40	2.66±0.79
72	3.47 ±0.89	3.41 ±0.91	1.73±0.29	2.98±0.78	2.90±0.88
96	3.92 ± 0.77	3.87 ±0.85	2.28±0.96	3.41±0.95	3.52±0.72
120	4.73 ±1.04	4.63 ±0.50	3.15±0.72	3.60±1.09	3.96±1.00
144	5.46 ±0.90	5.84±1.08	3.48±0.41	4.52±1.21	4.25±1.21

Table 2: Leaf lamina length of de-etiolated seedlings of A. viridis

Duration of light (hrs)	Length of leaf lamina (cm)				
	Dark	Red	Blue	Green	White
24	0.241 ±0.02	0.290±0.05	0.373±0.07	0.274±0.01	0.352±0.04
48	0.327±0.01	0.332±0.08	0.484±0.04	0.301±0.06	0.383±0.08
72	0.352±0.01	0.391±0.07	0.539±0.05	0.360±0.02	0.426±0.07
96	0.389±0.02	0.402±0.06	0.563±0.09	0.435±0.07	0.518±0.09
120	0.417±0.05	0.462±0.01	0.619±0.13	0.483±0.14	0.551±0.07
144	0.443±0.09	0.570±0.10	0.675±0.11	0.524±0.10	0.602±0.16

Table 3: Biomass production of de-etiolated seedlings of A. viridis

Duration of light (hrs)	Biomass Production(g)										
	Dark		Red		Blue		Green		White		
	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW	DW
48	5.041 ±0.57	0.204 ±0.045	5.000 ±0.32	0.221 ±0.070	5.092 ±0.48	0.381 ±0.032	5.032 ±0.49	0.150 ±0.029	5.047 ±0.790	0.292 ±0.055	0.292 ±0.055
96	5.016 ±0.82	0.611 ±0.021	5.084 ±0.84	0.847 ±0.081	5.031 ±0.79	0.604 ±0.017	5.015 ±0.27	0.374 ±0.053	5.038 ±0.84	0.680 ±0.070	0.680 ±0.070
144	5.007 ±0.91	0.728 ±0.050	5.049 ±0.49	1.734 ±0.036	5.064 ±0.56	1.362 ±0.035	5.033 ±0.98	0.648 ±0.067	5.062 ±0.0.75	1.161 ±0.093	1.161 ±0.093

Table 4: Chlorophyll a content of de-etiolated seedlings of A.viridis

Duration of light (hrs)	Chlorophyll a (mg/g)				
	Dark	Red	Blue	Green	White
24	0.036±0.004	0.036±0.005	0.057±0.001	0.045±0.004	0.059±0.001
48	0.033±0.008	0.090±0.007	0.130±0.005	0.132±0.07	0.331±0.03
72	0.004±0.003	0.175±0.03	0.200±0.06	0.224±0.04	0.365±0.02
96	0.018±0.007	0.245±0.07	0.204±0.08	0.341±0.05	0.352±0.01
120	0.035±0.001	0.221±0.06	0.396±0.09	0.407±0.07	0.380±0.01
144	0.029±0.001	0.319±0.02	0.344±0.05	0.380±0.01	0.449±0.02

Table 5: chlorophyll b content of de-etiolated seedlings of A.viridis

Duration of Light(hrs)	Chlorophyll b (mg/g)				
	Dark	Red	Blue	Green	White
24	0.013±0.001	0.011±0.003	0.012±0.001	0.017±0.001	0.22±0.08
48	0.012±0.001	0.082±0.005	0.037±0.0020	0.034±0.005	0.124±0.04
72	0.027±0.001	0.065±0.007	0.044±0.002	0.176±0.06	0.095±0.007

96	0.0087±0.0002	0.107±0.09	0.054±0.004	0.154±0.07	0.059±0.001
120	0.0025±0.0004	0.057±0.04	0.085±0.007	0.137±0.02	0.049±0.002
144	0.0089±0.0006	0.051±0.003	0.077±0.009	0.183±0.03	0.019±0.003

Table 6: Total chlorophyll content of de-etiolated seedlings of A. viridis

Duration of light (hrs)	Total chlorophyll content (mg/l)				
	Dark	Red	Blue	Green	White
24	0.049±0.004	0.047±0.004	0.079±0.005	0.062±0.002	0.081±0.006
48	0.045±0.007	0.378±0.06	0.160±0.07	0.167±0.09	0.455±0.07
72	0.027±0.003	0.217±0.09	0.241±0.08	0.400±0.15	0.460±0.05
96	0.025±0.002	0.392±0.05	0.255±0.09	0.406±0.08	0.410±0.10
120	0.038±0.005	0.318±0.10	0.501±0.14	0.434±0.16	0.452±0.86
144	0.039±0.008	0.398±0.08	0.421±0.11	0.316±0.06	0.463±0.98

Light reversed hypocotyl elongation as expected. Hypocotyl elongation was found to be inhibited in both the plants treated with blue LED light. Red LED light failed to reverse hypocotyl elongation in A.viridis. The expansion of the leaf lamina was found to be maximum in blue LED light and minimum in plants treated under dark condition. Under etiolated condition, leaf blades neither expanded nor unfolded. Dry matter production was found to be maximum in plants treated with red LED light. In A. viridis, chlorophyll a was found to be maximum in white LED light whereas, Chlorophyll b was found to be maximum in seedlings treated under green light. Total chlorophyll was found to be the maximum in seedlings irradiated with white light. In all the cases chlorophyll production was found to be least in etiolatedcondition.

4. Conclusion

The work was an attempt to investigate photomorphogenic response of A. viridis on the basis of its growth parameters such as hypocotyl elongation, length of leaf lamina, biomass production as well as on chlorophyll production**.** A complex network of molecular interactions couples the regulatory photoreceptors to developmental decisions. Hence further studies would be required to establish the enzymatic and molecular mechanisms involved in the changes of photomorphogenic responses under various wavelengths of light and darkconditions.

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