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INFLUENCE OF RED LIGHT ON SEED LIPASE DEPENDS ON THE LIPID CONTENT OF THE SEED

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ABSTRACT

Influence of blue, green, yellow, orange, red and white lights on lipase activity of *Oryza sativa* L. (Jyothi), *Vigna unguiculata* (L.) Walp. (Kanakamani) and *Arachis hypogaea* L. (CO-3) seedlings was analysed and it showed plant-wise variations. Considering the initial 7 days of development of the seedlings, green light for *O. sativa,* yellow light for *V. unguiculata* and red light for *A. hypogaea* were more influential in inducing lipase activity. In *A. hypogaea*, lipase activity was significantly higher under red light than other wavelengths from 2nd to 5th day which is the active period of seedling establishment. In *Oryza sativa* red light induced significant variation only on certain days. In *Vigna unguiculata* red light seemed to be least effective in stimulating lipase activity except on the first day. Thus effect of red light on lipase activity is species dependent and arguably, it depends on the lipid content of the seed. Blue wavelength exposure depressed the lipase activity in all the three seedlings studied.

Keywords: Llipase, light, red light, germination.

INTRODUCTION

The regulatory processes occurring during the degradation of reserve food materials of seeds have received greatly increasing attention. Knowledge of the regulation of metabolism by environmental factors enable us to modify plant metabolism for our advantage. Light is a very important environmental stimulus for plants, which controls the growth and development. Plants respond to a broad spectrum of light, ranging from UV-B to far-red light. A large body of physiological, photo-biological and molecular genetic studies have demonstrated that plants possess distinct photoreceptors sensing UV-B, UV-A, blue, green, red and far-red lights (Fankhauser and Chory, 1997). The photo-responses observed on the morphological, structural and metabolic levels, have an equivalent counterpart on the level of enzyme activities. Photomorphogenic responses are more or less direct result of specific control of enzymes. There are many enzymes, which were reported to be controlled by light (Schopfer, 1977; Vassey, 1988; Dreier *et al*., 1995). Light controls enzymes either by regulating the genes synthesizing it (Tobin and Silverthorne, 1985; Thompson and White, 1991) or by post-translational modifications (Buchanan, 1980).

In recent years great interest has arisen in the regulatory effect of light on different enzymatic activities (Darbelley *et al*., 1997; Datta *et al.,* 1999; Barnaby *et al*., 2004). But comparatively fewer studies were done on the effect of light on lipases. Lipases are enzymes that hydrolyze triglycerides. Many seeds contain triglycerides as major food reserve for germination. Red and white light treatments were found to have promotive effect on lipase activity in *Glycine max* while far-red light and dark treatments were inhibitory (Mehta *et al*., 1975). Similar result was obtained for seeds of *Cucumis sativus* and far-red light was found to reverse the effects of red light, indicating the involvement of phytochrome in the control of lipase activity. It was also suggested that the influence of light on lipid degradation is species dependent (Davies *et al*., 1981). Promotive effects of red and white light were obtained for germinating spores of the fern, *Anemia phyllitidis* (Gemmrich, 1982). Light- regulation of lipase activity was reported in seedlings of sunflower also (Pfeiffer and Kutschera, 1997). Pulsed concentrated solar radiation (Zodape and Chauhan, 1994) and UV-B radiations (Lo *et al*., 2004) were also found to enhance lipase activity.

The classical studies on the effect of light on plants revealed the importance of red, far red and blue lights in photomorphogenic responses. So most of the studies were concentrated on these spectral regions and other regions were considered to be ineffective for a long time. Very few studies were done on the effect of other wave lengths, especially in the metabolic level. But in recent years attention was given to other regions of the spectrum and

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recent evidences show that green light also has discrete effects on plant biology, and the mechanisms that sense this light quality are now being elucidated (Folta and Maruhnich, 2007). Many of the responses induced from the green portion of the spectrum are counterintuitive, often opposing normal light effects (Ahmad *et al*., 1998; Frechilla *et al*., 2000; Talbott *et al*., 2002; Eisinger *et al*., 2003; Folta, 2004; Dhingra *et al*., 2006; Bouly *et al*., 2007). Lipids are the major storage material of many seeds and so regulation of lipid metabolism during the germination and seedling establishment needs special attention. A very recent study suggests that green, red, and red/far-red light interact with blue light and contribute to the regulation of phosphorlipase A_2 α and β gene expression in *Citrus sinensis* (Liao and Burns, 2010). The present study tries to find out the role of different spectral regions in the regulation of the lipid hydrolyzing enzyme, lipase during seed germination and seedling establishment in three different plants- *Oryza sativa* L. (Jyothi), *Vigna unguiculata* (L.) Walp. (Kanakamani) and *Arachis hypogaea* L. (CO-3).

MATERIALS AND METHODS

Plant material and growth conditions

Three types of seeds which differ in their reserve food materials were selected for study: *Oryza sativa* L. (Jyothi), *Vigna unguiculata* (L.) Walp. (Kanakamani) and *Arachis hypogaea* L. (CO-3).

The above seeds were imbibed in distilled water for 12h under darkness. They were allowed to germinate and grow in controlled chambers with 12h light/ 12h dark photoperiod at 30± 2°C. Each chamber was provided with monochromatic light from arrays of 80-90 light emitting diodes having different wavelengths; Blue (peak wavelength 470 nm), Green (peak wavelength 520 nm), Yellow (peak wavelength 590 nm), Orange (peak wavelength 610nm) and Red (peak wavelength 660nm) with intensity, 1μmol $m^{-2}s^{-1}$. Controls were grown in a similar chamber, which was exposed to light from fluorescent tubes. The intensity of light was made similar to monochromatic light by paper filters.

After imbibition, seeds were given an initial 12h of light period followed by 12h of dark period. This cycle was continued for seven days and samples were collected immediately after the dark period. Seedlings were watered every day and sample populations were taken at 24h intervals for the initial seven days. Samples were collected immediately after the imbibition to determine the enzyme activity on zeroth day.

Preparation of enzyme extracts

A known quantity of sample was homogenized with twice the volume of ice-cold acetone. The crude homogenate was filtered through Whatman No. 1 filter paper. The powdered filtrate was washed successively with acetone, acetone: ether (1:1, v/v) and ether. The powder was air-dried and 1g of the powder was extracted in 20 ml of ice-cold phosphate buffer (50mM; pH 7.0). It was centrifuged at 15,000rpm for 10min at 4°C and the supernatant was used as enzyme source.

Enzyme assay

Lipase activity was determined by estimating the liberated fatty acid (Jayaraman, 1981). Substrate for the assay was prepared by adding 2ml of olive oil to 25ml of water. It was emulsified by adding 100mg of sodium taurocholate and 2g of gum accasia.

For assay, 20ml of the substrate was taken in a beaker and 5ml of phosphate buffer was added. It was stirred slowly, maintaining the temperature at 35°C. The pH was adjusted to 7.0. and 0.5ml of the enzyme extract was added and the pH was recorded immediately. At frequent intervals the pH was noted and 0.1 N NaOH was added to bring the pH to the initial value. From the volume of consumed NaOH, the released quantity of acid was calculated at discrete time intervals. One unit of enzyme activity was defined as the amount of enzyme, which releases one milli equivalent of free fatty acid per minute per gram sample. The data were statistically compared by one-way ANOVA for each day.

RESULTS

In order to determine the effect of different wavelengths on lipase, enzyme activity was determined for the first seven consecutive days. In all the three types of seeds tested, maximum lipase activity was seemed to be on the first day and gradually decreased thereafter with slight variations. The effect of different wavelengths on lipase activity showed variations in different plants and on different days.

Lipase activity in Oryza sativa seedlings grown under different wavelengths

Significant difference in enzyme activity between seedlings grown under different wavelengths was observed on first and second days and the greatest enzyme activity was observed in seedlings grown under green light (Table 1). However, on the following days, seedlings under other wavelengths showed greater enzyme activity (Table 1). But the differences between most of the wavelengths were found to be insignificant (Table 1). Seedlings under blue light showed the least enzyme activity on most of the days (Table 1). Considering the mean enzyme activity for seven days, seedlings under green light showed the greatest enzyme activity and seedlings under red, orange and yellow were close to control (Fig.1).

Table 1. Lipase activity (meq/min/g sample) in *Oryza sativa* seedlings grown under different-wavelength-lights.

Days	Different wavelengths					
	Blue	Green	Yellow	Orange	Red	Control (White)
0	0.0323±0.0003	0.0323±0.0003	0.0323±0.0003	0.0323±0.0003	0.0323±0.0003	0.0323±0.0003
1	0.0618±0.0008	0.1215±0.0015*$^{\bullet\clubsuit\blacklozenge\blacktriangledown\blacktriangle}$	0.0685±0.0005*	0.0994±0.0012*$^{\clubsuit\blacktriangle}$	0.0980±0.0010*$^{\clubsuit\blacktriangle}$	0.0718±0.0008*$^{\clubsuit}$
2	0.0404±0.0004	0.0742±0.0014*$^{\bullet\blacklozenge\blacktriangledown\blacktriangle}$	0.0626±0.0006*$^{\blacktriangledown\blacktriangle}$	0.0587±0.0027*$^{\blacktriangledown}$	0.0546±0.0016*	0.0574±0.0010*
3	0.0455±0.0050	0.0548±0.0018*$^{\blacklozenge\blacktriangle}$	0.0513±0.0009$^{\blacklozenge\blacktriangle}$	0.0441±0.0011	0.0551±0.0021*$^{\blacklozenge\blacktriangle}$	0.0441±0.0014
4	0.0305±0.0030$^{\bullet}$	0.0232±0.0012	0.0346±0.0016$^{\bullet}$	0.0317±0.0017$^{\bullet}$	0.0277±0.0027	0.0372±0.0012$^{\bullet\blacktriangledown}$
5	0.0382±0.0014$^{\clubsuit\blacklozenge}$	0.0367±0.0017$^{\blacklozenge}$	0.0334±0.0014$^{\blacklozenge}$	0.0259±0.0009	0.0350±0.0010$^{\blacklozenge}$	0.0351±0.0011$^{\blacklozenge}$
6	0.0264±0.0014	0.0262±0.0008	0.0331±0.0009*$^{\bullet}$	0.0300±0.0018	0.0404±0.0049*$^{\bullet\clubsuit\blacklozenge}$	0.0462±0.0013*$^{\bullet\clubsuit\blacklozenge}$
7	0.0280±0.0008	0.0316±0.0006	0.0235±0.0010	0.0228±0.0008	0.0276±0.0016	0.0292±0.0007

*enzyme activity significantly greater than that of seedlings under blue light. $^{\bullet}$ enzyme activitysignificantly greater than that of seedlings under green light. $^{\clubsuit}$ enzyme activity significantly greater than that of seedlings under yellow light. $^{\blacklozenge}$ enzyme activity significantly greater than that of seedlings under orange light. $^{\blacktriangledown}$ enzyme activity significantly greater than that of seedlings under red light. $^{\blacktriangle}$ enzyme activity significantly greater than that of control. meq - milli equivalents.

Table 2. Lipase activity (meq/min/g sample) in *Vigna unguiculata* seedlings grown under different-wavelength-lights.

Days	Different wavelengths					
	Blue	Green	Yellow	Orange	Red	Control (White)
0	0.0173±0.0003	0.0173±0.0003	0.0173±0.0003	0.0173±0.0003	0.0173±0.0003	0.0173±0.0003
1	0.0208±0.0008	0.0272±0.0007*	0.0294±0.0010*	0.0321±0.0011*$^{\bullet\clubsuit\blacktriangle}$	0.0405±0.0010*$^{\bullet\clubsuit\blacklozenge\blacktriangle}$	0.0279±0.0009*
2	0.0217±0.0007$^{\bullet\blacklozenge\blacktriangledown}$	0.0162±0.0010$^{\blacktriangledown}$	0.0241±0.0009*$^{\bullet\clubsuit\blacklozenge\blacktriangledown}$	0.0150±0.0010$^{\blacktriangledown}$	0.0101±0.0006	0.0253±0.0007*$^{\bullet\blacklozenge\blacktriangledown}$
3	0.0199±0.0009$^{\bullet\blacklozenge\blacktriangledown}$	0.0163±0.0007$^{\blacklozenge\blacktriangledown}$	0.0180±0.0005$^{\blacklozenge\blacktriangledown}$	0.0113±0.0009$^{\blacktriangledown}$	0.0072±0.0010	0.0250±0.0005*$^{\bullet\clubsuit\blacklozenge\blacktriangledown}$
4	0.0100±0.0015	0.0200±0.0008*$^{\blacktriangledown\blacktriangle}$	0.0181±0.0011*$^{\blacktriangledown\blacktriangle}$	0.0190±0.0006*$^{\blacktriangledown\blacktriangle}$	0.0107±0.0007	0.0123±0.0013
5	0.0104±0.0045	0.0085±0.0009	0.0125±0.0010	0.0098±0.0008	0.0085±0.0007	0.0094±0.0010
6	0.0124±0.0009$^{\bullet\blacktriangledown}$	0.0071±0.0010	0.0172±0.0007*$^{\bullet\blacktriangledown}$	0.0134±0.0009$^{\bullet\blacktriangledown}$	0.0065±0.0005	0.0167±0.0010*$^{\bullet\blacktriangledown}$
7	0.0173±0.0008$^{\blacklozenge\blacktriangledown}$	0.0155±0.0013$^{\blacklozenge\blacktriangledown}$	0.0234±0.0012*$^{\bullet\blacktriangledown\blacktriangle}$	0.0117±0.0007$^{\blacktriangledown}$	0.0082±0.0007	0.0175±0.0009$^{\blacklozenge\blacktriangledown}$

*enzyme activity significantly greater than that of seedlings under blue light. $^{\bullet}$ enzyme activity significantly greater than that of seedlings under green light. $^{\clubsuit}$ enzyme activity significantly greater than that of seedlings under yellow light. $^{\blacklozenge}$ enzyme activity significantly greater than that of seedlings under orange light. $^{\blacktriangledown}$ enzyme activity significantly greater than that of seedlings under red light. $^{\blacktriangle}$ enzyme activity significantly greater than that of control. meq - milli equivalents.

Table 3. Lipase activity (meq/min/g sample) in *Arachis hypogaea* seedlings grown under different-wavelength-lights.

Days	Different wavelengths					
	Blue	Green	Yellow	Orange	Red	Control (White)
0	0.0103±0.0008	0.0103±0.0008	0.0103±0.0008	0.0103±0.0008	0.0103±0.0008	0.0103±0.0008
1	0.0315±0.0005	0.0315±0.0007	0.0416±0.0006*$^{\bullet\blacklozenge\blacktriangledown\blacktriangle}$	0.0342±0.0007*$^{\bullet}$	0.0333±0.0013	0.0354±0.0010*$^{\bullet}$
2	0.0181±0.0011	0.0275±0.0010*	0.0202±0.0016	0.0226±0.0063*	0.0302±0.0012*$^{\clubsuit\blacktriangle}$	0.0214±0.0014
3	0.0148±0.0008	0.0179±0.0009*$^{\blacktriangle}$	0.0187±0.0007*$^{\blacktriangle}$	0.0170±0.0010$^{\blacktriangle}$	0.0205±0.0005*$^{\blacklozenge\blacktriangle}$	0.0129±0.0009
4	0.0135±0.0010	0.0155±0.0007$^{\clubsuit}$	0.0112±0.0012	0.0238±0.0008*$^{\clubsuit\blacktriangle}$	0.0296±0.0006*$^{\clubsuit\blacklozenge\blacktriangle}$	0.0163±0.0013*$^{\clubsuit}$
5	0.0106±0.0006	0.0104±0.0004	0.0146±0.0009*$^{\bullet}$	0.0256±0.0004*$^{\clubsuit\blacktriangle}$	0.0239±0.0009*$^{\clubsuit\blacktriangle}$	0.0130±0.0010*$^{\bullet}$
6	0.0212±0.0012$^{\clubsuit\blacklozenge}$	0.0254±0.0009*$^{\clubsuit\blacklozenge\blacktriangle}$	0.0186±0.0006$^{\blacklozenge}$	0.0110±0.0010	0.0212±0.0007$^{\clubsuit\blacklozenge}$	0.0219±0.0008$^{\clubsuit\blacklozenge}$
7	0.0219±0.0009$^{\clubsuit\blacklozenge\blacktriangle}$	0.0194±0.0014$^{\clubsuit\blacklozenge\blacktriangle}$	0.0115±0.0010	0.0143±0.0008$^{\clubsuit\blacktriangle}$	0.0107±0.0007	0.0105±0.0005

*enzyme activity significantly greater than that of seedlings under blue light. $^{\bullet}$ enzyme activity significantly greater than that of seedlings under green light. $^{\clubsuit}$ enzyme activity significantly greater than that of seedlings under yellow light. $^{\blacklozenge}$ enzyme activity significantly greater than that of seedlings under orange light. $^{\blacktriangledown}$ enzyme activity significantly greater than that of seedlings under red light. $^{\blacktriangle}$ enzyme activity significantly greater than that of control. meq - milli equivalents.

Lipase activity in Vigna unguiculata seedlings grown under different wavelengths

On the 1st day, the greatest lipase activity was observed in *V. unguiculata* seedlings subjected to red light treatment. But later it showed less enzyme activity than seedlings grown under other wavelengths (Table 2). Greatest level of enzyme activity was observed for seedlings grown under white light on 2nd and 3rd days, under green light on 4th day and yellow light on 5th, 6th and 7th days (Table 2). The differences in enzyme activity between seedlings under most of the wavelengths were significant up to the 4th day. But on 5th day the differences in enzyme activity on exposure to various wavelengths were insignificant. On 6th and 7th days the enzyme activity increased slightly and the difference between some of them were significant (Table 2). Considering the mean enzyme activity for seven days, seedlings under yellow light showed the greatest enzyme activity than others, but close to control. Seedlings under red light showed the least enzyme activity (Fig. 2).

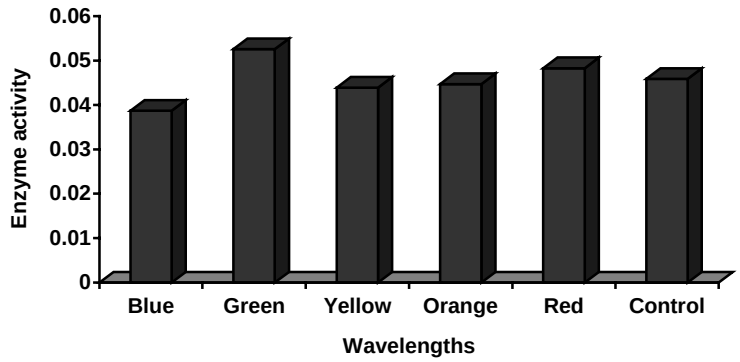

Fig. 1. Mean lipase activity for seven days of *Oryza sativa* seedlings grown under different-wavelength-lights.

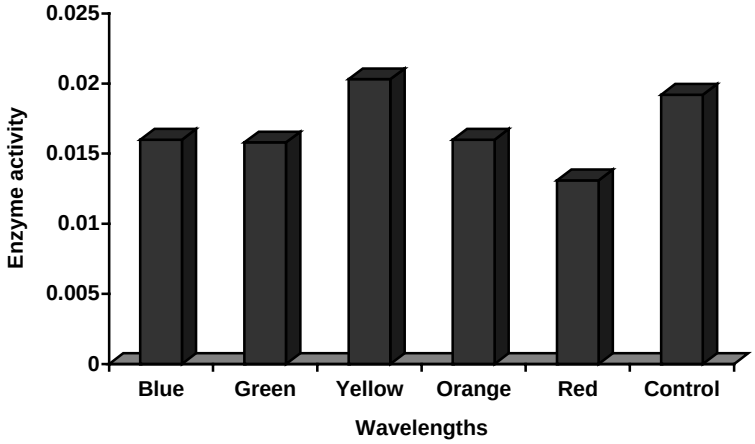

Fig. 2. Mean lipase activity for seven days of *Vigna unguiculata* seedlings grown under different-wavelength-lights.

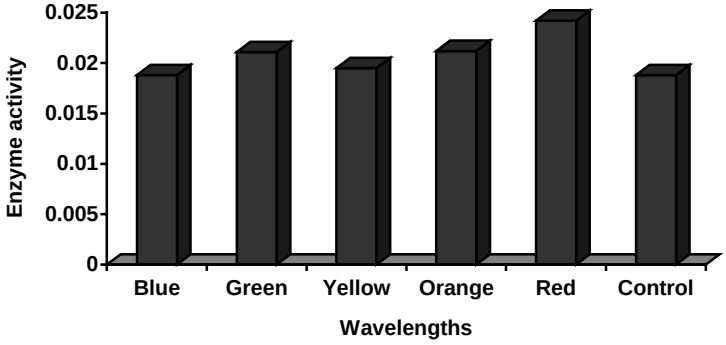

Fig. 3. Mean lipase activity for seven days of *Arachis hypogaea* seedlings grown under different-wavelength-lights.

Lipase activity in Arachis hypogaea seedlings grown under different wavelengths

On the 1st day, seedlings under yellow light showed significantly greater enzyme activity than others (Table 3). But on other days it showed less activity compared to others (Table 3). From the 2nd day onwards seedlings under red light showed significantly greater enzyme activity than most of the others (Table 3). But on 6th and 7th days it showed less activity (Table 3). Seedlings under blue light showed significantly less enzyme activity than seedlings under most of the other wavelengths up to 5th day. But on 6th and 7th days it showed significantly greater activity than some of the other wavelengths (Table 3). Seedlings under red light showed significantly greater

enzyme activity than control from 2nd to 5th day. But on 1st, 6th and 7th days the differences were insignificant (Table 3). Considering the mean enzyme activity for seven days, seedlings under red light showed the greatest enzyme activity than others (Fig. 3). Thus, during the active period of seedling establishment red light enhances lipase activity.

DISCUSSION

In all experiments conducted, maximum lipase activity was observed on the first day of germination and it decreased as the growth proceeded. Of the three types of seeds tested, *A. hypogaea* has the highest lipid content, but it didn't show greater lipase activity than others as we may expect. It is in accordance with the findings of Bamann and Ullman (1957) that, there is little correlation between lipid content and lipase activity. Though some earlier studies reported that there is no correlation between light and lipase activity (Nyman, 1965; Castlefranco *et al*., 1969; Bajracharya and Schopfer, 1979) some other studies revealed that lipase activity is induced by light (Mehta *et al*., 1975; Davies *et al*., 1981; Gemmrich, 1982; Pfeiffer and Kutschera, 1997).

According to the previous reports, red and white light induce lipase activity. Some recent studies reveal the importance of other wavelengths also in lipid metabolism (Liao and Burns, 2010). Present study was an attempt to find out the influence of different wavelengths of the spectrum in the lipid mobilization during seed germination and seedling establishment in three different seeds which differ in their reserve food material. The effect of different lights on lipase activity varied on different days and on different plants. It is observed that, for *O. sativa,* green light is more influential; for *V. unguiculata,* yellow light is more influential and for *A. hypogaea,* red light is more influential in inducing lipase activity in the initial 7days of the seedlings. In the case of *A. hypogaea,* seedlings under red light showed significant increase in lipase activity than others on most of the days. Mean values of the enzyme activity for seven days showed that, in *A. hypogaea* it was higher for seedlings under red light than others and in *O. sativa*, red-light-exposed seedlings came to the second place. But in *V. unguiculata*, seedlings undergone red light exposure, showed the least in enzyme activity. However, on the first day, which may be the most active period of lipid degradation, seedlings under red light showed significantly greater enzyme activity than others. In *A. hypogaea*, a major part of the reserve food is lipid. In *O. sativa* also oil content is high, but in *V. unguiculata* it is very less. The developmental pattern is also different in the above plant groups. In *V. unguiculata* and O. *sativa* it is faster than *A. hypogaea.* Davies *et al.* (1981) suggested that the effect of red light on lipase activity is species dependent. Present study agrees with it and suggests that influence of red light on lipase activity during seed germination and seedling growth depends on the lipid content of the seed as well. It also reveals that each part of the spectrum has its own effect on lipase activity and is also species dependent. The present study reveals the depressing effect of blue wavelength on lipase activity also.

CONCLUSION

Each wavelength of the visible spectrum affects the lipase activity during seed germination and seedling establishment. Effect of red light on lipase activity depends on the lipid content of the seed. In seeds having high lipid content, the lipase activity enhances under red light. The blue wavelength has a depressing effect on lipase activity.

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