1. INTRODUCTION

There are very few reports on tissue culture of amaranths (1,2,3,4). It was shown that Amaranthus paniculatus develops a highly proliferative callus in vitro on the B5 medium supplemented with 1 ppm each 2,4-Dichlorophenoxy acetic acid (2,4-D), kinetin (Kn) and gibberellic acid (GA3) and is therefore a good system to understand the biochemical basis of cell division and proliferation. The exact mechanism of cell proliferation and the role of light in cell proliferation in tissue culture is not known. The activity of the enzyme, glyoxalase-I (S-lactoyl GSH methyl glyoxalase EC 4.4.1.5) serves as an indicator of cell growth status. The enzyme converts \( \alpha \)-keto aldehyde into thiol ester of glutathione which is converted to \( \alpha \)-hydroxyl carboxylic acid by glyoxalase-II (2,5,6,7). The presence of the glyoxalase enzyme in plants has been reported (6) and a correlation was found between an increase in the enzyme activity with increased callus growth (7,8,9). In this paper we have attempted to study the role of light in cell proliferation and to check if glyoxalase activity is also stimulated by light.

2. PROCEDURE

2.1 Plant Material: The seeds of Amaranthus paniculatus were obtained from Indian Agricultural Research Institute, Delhi. Seeds were surface sterilized with 0.1% \( \text{HgCl}_2 \) and germinated on B5 medium (10) containing 0.8% agar and 2% sucrose without any hormones. Hypocotyl segments from 10 days old plantlets were inoculated on B5-medium (10) supplemented with 1mg/litre each of kinetin, gibberellic acid and 2,4-D. After 15 days the callus was subcultured on the same medium or the medium under the experimental condition and maintained at 25±1°C under constant illumination (1200 \( \mu \text{W Cm}^{-2} \)) and in darkness.

2.2 Light treatments: Light treatments were given to the different calli as per the experimental conditions. Red light (0.066 \( \mu \text{W Cm}^{-2} \)) was obtained by filtering the light from four 100 W tungsten lamps through a CBS-650 filter.
Far-red light (0.045 pW cm\(^{-2}\)) was obtained from a 250 W tungsten reflector lamp (Westinghouse, USA), the output of which was filtered through a CBS-750 filter (Carolina Biological Supply Co., USA); blue light (0.0015 pW cm\(^{-2}\)) was obtained from 8.40 watts fluorescent tubes (Philips, India), light filtered through a CBS-450 filter. The growth (fresh weight) of calli was measured after 8 days of culture. The calli were transferred to darkness for 4 days and on the 5th day after the required light treatments of red, red+far-red and blue light, glyoxalase was assayed, after 4 hrs, 12 hrs and 24 hrs respectively. The experiments were repeated at least twice and ± SD was calculated.

2.3 Glyoxalase assay: The enzyme glyoxalase-I (S-lactoyl GSH methyl glyoxalase EC 4.4.1.5) was extracted by homogenizing callus (1 gm) in 0.1M sodium phosphate buffer (pH 7.0) containing 20 mg MgSO\(_4\)\(\cdot\)7H\(_2\)O and 20 ml glycerol/100 ml. The extract was centrifuged at 15000 rpm for 20 min. in SS34 rotor in Sorvall RC5 centrifuge. The supernatant was fractionated with 40-80% ammonium sulfate and the precipitate was re-dissolved in the extraction buffer, and employed for glyoxalase-I assay according to Racker (5) and as modified for plant tissues by Ramaswamy et al. (7). The assay mixture contained 100 mM sodium phosphate buffer (pH 7.5), 3.5 mM methyl glyoxal, 15 mM sodium phosphate buffer (pH 7.5), 35 mM methyl glyoxal, 15 mM magnesium sulfate and 1.7 mM glutathione. The mixture was incubated for 7 min for formation of a complex between methyl glyoxal and glutathione. The reaction was started by the addition of enzyme (0.02 ml). The formation of thioester which shows maximum absorption at 240 nm was measured in Shimadzu UV-260 spectrophotometer. The enzyme unit (IU) is defined as the amount of enzyme catalyzing the formation of 1 \(\mu\)mol of S-lactoylglutathione from methyl glyoxal and reduced glutathione/min. at 25°C. Specific activity of the enzyme was determined as units per gm. fr. wt.

3. RESULTS AND DISCUSSION

To check the effect of light on proliferation, callus growing on B5+2,4-D+GA\(_3\)+Kn was transferred to B5 Basal medium, (minus hormones) or to the same medium but kept in either total darkness or continuous light regime or treated under different light treatments of red, red+far-red and blue light.
3.1

Fig. 1(a) Cell proliferation in light and dark in *Amoranthus paniculatus* callus maintained in all three hormones or transferred from medium containing all three hormones to Basal medium. Cell proliferation expressed as gm. fr. wt.

Fig. 1(b) Effect on glyoxalase-I activity in callus grown on B5 (minus hormones) and medium containing all 3 hormones under light and dark condition. After 8 days of culture the glyoxalase-I activity was measured and expressed as units gm. fr. wt.

As shown in Fig. 1(a) after 8 days of culture the callus showed greater proliferation in light grown cultures. It was observed that hypocotyl segments when grown on B5 medium minus hormones showed very slow proliferation and in turn showed high proliferation when grown with all 3 hormones, indicating that hormones triggered this response. In the present study it seems that light has an effect over and above that induced by hormones. There was an 175% increase in cell proliferation in light over their corresponding dark grown cultures. If the cultures were left in darkness for long, the proliferation reduced further.

3.1.1 The cell proliferation has been related to the glyoxalase-I activity (2,6,7,8,11). Since we have shown earlier, correlation between glyoxalase activity and proliferation (2,7,8), in the present study we checked, if light also plays a role in glyoxalase activity. As evident by the data in Fig. 1(b) it was observed that glyoxalase activity was higher in all 3 hormones when compared to B5 basal medium but there was a 155% increase of glyoxalase activity in light grown cultures in all 3 hormones when compared to their corresponding dark controls. The mechanism by which light enhances the enzyme activity remains to be seen, however it correlates
with cell proliferation as is evident in the present set of experiments.

3.2

![Graph](image_url)

**Fig.2(a)** Effect on glyoxalase-I activity in all 3 hormones in dark and 5 min of red light treatment in *Amaranthus paniculatus* cultures. The glyoxalase activity was measured after 4, 12 and 24 hours respectively on the 5th day. The glyoxalase activity was expressed as units sp. activity/gm. fr. wt.\(^{-1}\) Fig.2(b) Percentage increase in glyoxalase activity at 24 hrs, in red(R) far-red (FR), R+FR and R+FR+R light treated cultures over their corresponding dark grown cultures.

3.2.1. To check for the involvement of phytochrome in cell proliferation, the cultures were exposed to red light irradiation for 5 min and glyoxalase assay was done after 4 hr, 12 hrs and 24 hrs respectively. At 4 hrs there was a 162% increase in stimulation of glyoxalase activity compared to the dark controls and also increase in stimulation was observed in 12 and 24 hrs as shown in Fig.2(a). To check if this stimulatory effect was under phytochrome control red light and far-red light reversible experiments were done which usually demonstrate low energy response of phytochrome action. \((13,14)\). As shown in Fig.2(b) there was no clear R/FR reversibility observed. Infact far-red alone showed 187% increase and the red light stimulation of 250% was not reversed by FR light. It seems that stimulation of glyoxalase activity could be a very low fluence response. Also it is not clear why in R+FR+R there was a further decrease in enzyme activity. This needs to be studied in detail.
3.3

Effect on glyoxalase-I activity in all 3 hormones in dark and 5 min of blue light irradiation.

3.3.1 Further experiments were done to check if blue light was involved in stimulating glyoxalase activity. For this cultures were exposed to blue light treatment for 5 min and glyoxalase activity was measured after 4 hrs, 12 hrs and 24 hrs respectively. At 4 hrs there was a 278% increase in stimulation in glyoxalase activity over the dark cultures, whereas there was only 162% increase by red light. It is known that various responses are induced in plants that are operated by blue light activity via phytochrome or its own photoreceptor (12,15). It would be interesting to check in this system if the effects of blue light on the stimulation of glyoxalase is mediated via phytochrome, or a blue light receptor.

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5. REFERENCES