Fig. 1  The reaction catalyzed by glyoxalase-I and glyoxalase-II to convert methyl glyoxal to lactic acid.
Spontaneous Glyoxalase I

\[
\text{CH}_3 \quad \text{CO} + \text{GSH} \rightleftharpoons \text{CO} \quad \text{HCOH} \quad \text{HCOH} + \text{GSH}
\]

Glyoxalase I

\[
\text{CH}_3 \quad \text{HCOH} \quad \text{HCOH} \quad \text{CO} \quad \text{COOH}
\]

Hemi-mercaptal

\[
\text{CH}_3 \quad \text{SG} \quad \text{SG} \quad \text{SG}
\]

S-lactoyl GSH

Glyoxalase II

Methyl glyoxal

\[
\text{HCO} \quad \text{HCOH} \quad \text{HCOH} \quad \text{HCOH}
\]

D-lactic acid
Fig. 2 Seeds obtained from plants grown in the field (A) were germinated on agar+sucrose medium. 10 day old seedlings (B) were used to get the hypocotyl as an explant which was cultured on B₅+Kn+2,4D+GA₃ (1 ppm each) medium to get callus (C).
PLANT
AMARANTHUS PANICULATUS
seedling
CALLUS
(B5+Kn+2,4-D+GA3)
Fig. 3  Emission spectrum of blue light filter (Carolina Biological Supply Co., USA) using Licor-1800 spectroradiometer.
Fig. 4  The IP$_3$ standard curve from which the IP$_3$ content, IP$_3$ pmole/tube was calculated.
$\frac{B}{B_0}$ vs. $IP_3$ pmole/tube

IP$_3$ Standard Curve
Fig. 5 Callusing in different explants on B5+2,4D+GA3+Kn (1ppm each) medium. A total of sixty cultures were maintained for each explant. The data is recorded after 40 days of culture.
Fig. 6 Selection of different cell lines from mixed callus obtained from hypocotyl explant. The callus lines producing either amaranthin, chlorophyll or no pigment at all were selected. The white callus line showed higher growth and was used for cell proliferation studies.
Fig. 7  Effect of light on cell proliferation in callus cultures grown on B5 basal medium and B5 hormone medium. The final weights of the calli were taken on the 9th day.
Fig. 8  Effect of light on cell numbers in suspension cultures grown in B5 basal medium and B5 hormone medium. The cell counts were taken on the 9th day.
Fig. 9 Kinetics of light stimulated cell proliferation. Callus growing in an hormone medium was sub cultured and kept in light or in darkness and the recordings were taken everyday. For each treatment a minimum of twenty replicates were kept.
Fig. 10 Kinetics of red light stimulated cell proliferation. Callus growing on hormone medium was sub cultured and kept in darkness and a pulse of 5 min. of red light per day was given for 9 days. The recordings were taken every day. For each treatment a minimum of twenty replicates were kept.
Fig. 11 Involvement of phytochrome in light mediated cell proliferation. Callus was grown in darkness, and each day red and/or far-red light treatment for 5 min was given. The values were recorded on the 9th day of culture, for each treatment twenty replicates were kept.
Fig. 12 Demonstration of presence of phytochrome I by ELISA in extracts obtained from callus grown in white light. A: value obtained using control ascites antibody B: value obtained using a monoclonal antibody against phytochrome I.
Fig. 13  Original tracing of phytochrome measurement performed with *Brassica* seedling and *Amaranthus* callus after R and FR irradiation. The spectrophotometer was set at dual wavelength mode and the two wavelengths were set at 660 nm and 730 nm. The $A_{660} - A_{730}$ of the sample was recorded after 1 min. R and 1 min. FR irradiation alternately.
Fig. 14 Kinetics of blue light stimulated cell proliferation. Callus growing on hormone medium was sub cultured and kept in darkness and a pulse of 5 min. of blue light per day was given for 9 days. The recordings were taken every day. For each treatment a minimum of twenty replicates were kept.
Fig. 15 Effect of different concentrations of methyl glyoxal on cell proliferation in callus culture grown on hormone medium. The recordings were taken on fifth day of culture.
Fig. 16 Effect of different concentration of MG on cell numbers during cell proliferation. Suspension cultures were maintained in hormone medium. The data were taken on fifth day.
Fig. 17 Effect of methyl glyoxal (0.5 mM) on radio-labelled thymidine incorporation into TCA insoluble precipitate. A ratio of thymidine incorporated to its total uptake is given.
Fig. 18 Activity staining for glyoxalase-I in 'crude extract of' *Amaranthus* callus and purified glyoxalase obtained from *Brassica*.

1. Crude extract
2. Purified glyoxalase.
Fig. 19 Effect of light on glyoxalase I activity. The callus was grown both on basal medium (minus hormones) and hormone medium and cultures were kept both in light and darkness. Glyoxalase assay was done on 9th day and activity was recorded as IU gm. fr. wt.$^{-1}$
Fig. 20 Kinetics of red light stimulated glyoxalase I activity. Red light irradiation was given for 5 min. to callus grown on hormone medium in darkness. The enzyme activity was measured at 4, 12 and 24 hours and recorded as IU gm. fr. wt. \(^{-1}\).
Fig. 21 Involvement of phytochrome in light stimulated glyoxalase activity. R (5 min.), F+FR (5 min. each), FR (5 min.) R+FR+R (5 min each) were given to five day old sub cultures grown on hormone medium in darkness. After each irradiation glyoxalase assay was done at 4, 12, and 24 hours respectively. The enzyme activity is given as IU gm. fr. wt. -1.

Fig. 22 Involvement of phytochrome in light stimulated glyoxalase I activity. Other experimental details are exactly similar to those mentioned in legend of above figure except that Glyoxalase I activity is expressed as IU mg protein -1.
Fig. 23 Glyoxalase I activity in FR, R, R+FR and R+FR+R. The values taken from 24 hour treatment are replotted as percent increase over dark control.
Involvement of phytochrome in light stimulated glyoxalase I activity. R (5 min.), FR (5 min.), R+FR (5 min. each) and R+FR+R (5 min. each) were given every day for 8 days to callus cultures grown on hormone medium. Cultures otherwise were kept in darkness. Enzyme assay was done on the ninth day and activity is given as IU gm.fr.wt.\(^{-1}\).

Fig. 24

Involvement of phytochrome in light stimulated Glyoxalase I activity. Other experimental details are exactly similar to the legend of the above figure except that enzyme activity is given as IU mg Protein\(^{-1}\).

Fig. 25
Fig. 26 Kinetics of blue light stimulated glyoxalase I activity, blue light irradiation was given to callus grown on hormone medium in darkness. Enzyme activity was measured at 4, 12 and 24 hours and recorded as IU mg protein$^{-1}$. 
Fig. 27 Kinetics of increase in glyoxalase-I activity after irradiation of callus cultures with blue light for 5 min.
Fig. 28  Kinetics of Ca\textsuperscript{2+} uptake as affected by white light, red light and blue light. The cultures were grown for 5 days on hormone medium without calcium. After irradiation, uptake and incorporation of labelled calcium was studied at different time intervals.
Fig. 29  Elution profile of the standards, GPI, GPIP and GPIP$_2$ from Dowex-50.

GPI, GPIP and GPIP$_2$ obtained from Sigma were applied onto formate anion-exchange columns and eluted sequentially with:

a. Distilled water (fractions were not collected).

b. 5 mM NaBaO$_7$/0.18M NH$_4$ formate for GPI;

c. 0.1M formic acid/0.3M NH$_4$ formate for GPIP; and

d. 0.1 ml formic acid/0.75 M NH$_4$ formate for GPIP$_2$.

1 ml fractions were collected and inorganic phosphate (Pi) was estimated as described in "Materials and Methods".
Fig. 30A&B Separation of GPI, GPIP and GPIP$_2$ obtained after deacrylation of [32P] inositol phosphoinositide from proliferating Amaranthus callus grown in Dark (Fig. 30A) and in white light (Fig. 30B). 5 day old callus cultures were incubated with [32P] orthophosphate 60 μCi/ml and the phosphoinositides were extracted and deacylated. The water-soluble deacylated products, glycerophosphoinositides, were applied onto formate anion-exchange columns and sequential elution was done as described in "Materials and Methods".

1 ml fraction were collected and counted for radioactivity as described in "Materials and Methods".

Each experiment was repeated at least thrice and the data presented is the mean of result obtained.
Fig. 31  Comparison of the levels of PI, PIP and PIP$_2$ separated through TLC. 5 day old callus cultures were incubated with $^{32}$P orthophosphate (60 µCi/ml) and after different light treatments of R 1 min. and B 1 min, the samples were run on TLC.
Fig. 32  Effect of 5 min. irradiated red light and blue light on the turnover of PI, PIP and PIP₂. The callus was incubated with \(^{32}\)P orthophosphate (60 μCi per ml) and after four hours of incubation pulses of red light and blue light were given separately for 5 min. and the phosphoinositides were extracted after 1 min. and 5 min. respectively and were separated through TLC. Figure A shows separation and turnover after 5 min of light treatment. Figure B shows the separation and turnover after 1 minute of light treatment. The spots were quantified by scanner and area under the peak plotted.
Fig. 33 Measurement of IP$_3$ in calli irradiated with red and blue light for 1 min and 5 min. IP$_3$ was measured using Amersham D-myo-inositol 1,4,5-triphosphate (IP$_3$) ($^3$H) assay system.
Fig. 34 Effect of lithium and calcium ionophore A23187 on cell proliferation. The readings were taken after 9 days of culture. Lithium and A23187 were used along with B5 hormone medium at 20 mM and 20 μM concentrations respectively.
Fig. 35: Effect of lithium and calcium ionophore A23187 on glyoxalase I activity. The readings were taken after 9 days of culture. Lithium and A23187 were used along with B5 hormone medium at 20 mM and 20 μM concentrations respectively.
Fig. 36 Effect of hormones and light on cell proliferation. Callus growing on B5 basal medium was transferred to B5 medium containing Kn, 2,4D or GA3 either individually or all three together and kept in darkness or light. The values were recorded after 9 days of culture.
Fig. 37 Effect of hormones and light on glyoxalase I activity. Callus growing on B5 basal medium was transferred to B5 medium containing Kn, 2,4D or GA₃ either individually or all three together and kept in darkness or light. The values were recorded after 9 days of culture.
Fig. 38 Effect of light on cell proliferation in callus cultures transferred from B5 hormone medium to B5 with individual hormones. The data were recorded after 9 days of culture.
Fig. 39 Effect of light on glyoxalase I activity in callus cultures transferred from B5 hormone medium to B5 with individual hormones. The data were recorded after 9 days of culture.
Fig. 40  Comparison of the growth of a callus line, (A) growing on hormone medium with fast proliferative callus line (B) that was selected through repeated suspension cell cultures. The picture is taken after 8 days of culture. Callus line that was selected in 1988 was retested for its proliferation in 1989.
Fig. 41  Comparison in fast proliferating cell line (FPCL) and slow proliferative cell line (SPCL). The data were recorded for 4 subcultures.
Fig. 42A&B Direct regeneration of shoots (A) and shoots/Roots (B) from hypocotyl explant. The hypocotyl was cultured on MS+Kn (0.5 ppm) + NAA (0.1 ppm) in case of A and on MS + NAA (0.2 ppm) + Kn (3 ppm) in case of B and regeneration was noted after 20 days of culture. In (A) hypocotyl also show callusing from one side.
Fig. 43A&B Direct regeneration of roots from hypocotyl explant. The hypocotyl was cultured on medium MS basal 1/2 + NAA (1 ppm) + 1/10th myoinositol + 0.1% activated charcoal. The regeneration was noted after 20 days of culture. In A: the regenerated roots showed multiple root hairs and in B: on further subculturing in the same medium, the elongation of roots was seen.
Regeneration of secondary inflorescence, vegetative structures and plantlets from immature floral buds. The floral buds of the cultured immature inflorescence showed two types of responses in the presence of cytokinins supplemented MS medium.

A. a typical bright green inflorescence.

B. 30 day old immature inflorescence showing formation of secondary inflorescence from floral buds in MS+5 mg/l Kn.

C. Multiple shoots from activated floral buds on MS+10mg/l Kn.

D. Formation of leafy structures in activated floral buds subcultured on MS+10 mg/l BAP. Callus was also induced at the base.

E. Development of plantlets on MS+15% CM + 12mg./l Kn.
Fig. 45A&B  Effect of cytokinins on the regeneration of secondary inflorescence, leaves and shoots from immature inflorescence explants of *Amaranthus paniculatus*. A: Effect of Kinetin at 1-16 mg/l. B: Effect of BAP at 1-10 mg/l. The dotted lines gives the total percent of cultures responding.
Fig. 46 Effect of different concentrations of NAA on root formation from callus obtained from hypocotyl segments. For each treatment twenty replicates were kept.
Differentiation of roots from hypocotyls callus.

A. Roots growing in callus on MS1/2 + NAA (0.2 ppm) + 1/10th myoinositol medium.

B. Roots growing in callus on MS1/2 + BAP (0.5 ppm) + 1/10th myoinositol medium.

C. Multiple roots in callus grown on MS1/2 + NAA (0.2 ppm) + 0.1% activated charcoal medium.

D. Multiple roots in callus grown on MS1/2 + BAP (0.5 ppm) + CM 15% + 8 μM picloram.
Differentiation of shoots from hypocotyl callus.

A. Initiation of shoots in callus grown in MS1/2 basal + NAA (0.2 ppm) + BAP (2 ppm) + 1/10th myoinositol medium. and

B. on MS1/2 basal + 1AA (0.3 ppm) + Kn (3 ppm) + 1/10th myoinositol.

C. Formation of embryoid like structures on MS1/2 basal + CM 15% + Zeatin (0.5 ppm) medium, with anthocyanin production in the callus also.

D. Formation of embryoid like structures on MS1/2 basal + CM 15% + Zeatin (0.5 ppm) medium. No anthocyanin production is observed here.
Fig. 49 Methyl glyoxal-glyoxalase model.

To explain how the level of MG which is an inhibitor of cell division, can be regulated in two ways. Either the machinery regulating the synthesis of MG is to be regulated or the machinery degrading the MG (detoxification mechanism) is to be regulated. Light could increase cell proliferation by decreasing the MG level either via inhibiting the synthesis of MG or by increasing glyoxalase-I activity. In the present work light was found to increase glyoxalase-I.
PHOTORECEPTOR $\rightarrow$ Signal $\rightarrow$ Glyoxalase I

(Phytochrome or Cryptochrome)

$\rightarrow$ Methylglyoxal

Synthesis $\rightarrow$ Degraded

$\rightarrow$ Cell Proliferation
Fig. 50 A model suggesting the possible role of phosphoinositide cycle, calmodulin and glyoxalase in cell proliferation. The model proposes that the alteration in phosphoinositide levels could lead to an increase in cytoplasmic calcium which would activate the enzyme glyoxalase-I through the involvement of calmodulin. Glyoxalase-I in turn would regulate cell proliferation. The increase in cytoplasmic calcium could be through release from endogenous pool or by enhanced uptake from outside the cell.
Endogenous pool of Ca$^{2+}$

Ca$^{2+}$ in

Cam Inactive

Cam Active

Glyoxalase I active

Cell proliferation
The model shows the possible sites of light action, signal transduction and the mode of glyoxalase-I increase. It proposes that light is perceived by either phytochrome or cryptochrome or both. After excitation or getting converted into an active form, the pigment, through a still unknown mechanism, results in an enhanced turnover of phosphoinositol lipids. The initial site of action seems to be different for B and R. The initial action of B seems to be at the level of PIP, leading initially to an increase in PIP<sub>2</sub> level which would be degraded later. In R PIP<sub>2</sub> is also degraded resulting in production of IP<sub>3</sub> and DAG. PI turnover would increase Ca<sup>2+</sup> level which in turn would activate the glyoxalase-I through an involvement of calmodulin.
glyoxalase Inactive → glyoxalase Active