SUMMARY

The results obtained on the role and mechanism of light stimulated cell proliferation in callus cultures together with attempts to regenerate plants in \emph{in vitro} cultures in \textit{Amaranthus paniculatus} are summarized below:

1. A callus line was selected which proliferated at higher rate in the presence of light and at the same time did not develop chlorophyll. This line was used to study photomorphogenetic effects of light in the regulation of cell growth and proliferation.

2. The stimulation of cell proliferation by light did not occur in absence of hormones. A basic level of hormones (1 ppm each of 2,4-D, GA$_3$ and Kn) was required to elicit the response. Light effects were over and above those obtained by hormones.

3. The cell proliferation as affected by light in callus cultures was reflected in increase in cell numbers.

4. To check the involvement of phytochrome and/or blue light photoreceptors, callus was irradiated with lights of different wavelengths, red (R) ($\lambda_{\text{max}}$ 660 nm), far-red (FR) ($\lambda_{\text{max}}$ 730 nm) and blue ($\lambda_{\text{max}}$ 430 nm). In
experiments where FR light was given after R light, no photoreversibility was observed. Infact in FR light alone, cell proliferation was stimulated. Short durations of blue light also showed stimulation in cell proliferation. The results strongly suggest that a blue light photoreceptor may be involved. Nevertheless the involvement of phytochrome cannot be totally ruled out. Infact there seems to be greater chances of the involvement of phytochrome II in callus cultures. A methylglyoxal glyoxalase model, hypothesised to explain the mechanism of cell proliferation, was tested. According to the model, an increase in MG level can block cell proliferation. Therefore, MG levels have to be strictly regulated in order to maintain cell division and proliferation. This can be achieved in two ways, either regulating the degradation or synthesis of MG (detoxification).

5. Methyl glyoxal the substrate for glyoxalase enzyme inhibited cell division, and there was inhibition both in fresh weight as well as in cell numbers. A 75% inhibition was obtained at 5 mM MG concentration. Methylglyoxal also inhibited DNA synthesis, as checked by $^3$H-thymidine incorporation. The incorporation of $^3$H-thymidine decreased in callus cells grown in the presence of MG. It is concluded that MG does inhibit
cell proliferation and DNA synthesis in the present system.

6. Experiments were done to check the presence of glyoxalase-I through activity staining of the enzyme on the gel. The presence of glyoxalase-I was shown in *Amaranthus* and its activity band on the gel coincided with pure enzyme obtained from *Brassica juncea*.

7. There was a positive correlation between cell proliferation by light and glyoxalase-I activity. The glyoxalase activity was 55% higher in callus grown in light on hormone medium compared to dark grown cultures. In B5 basal medium there was not much effect of light.

8. To check for the involvement of phytochrome in light stimulation of glyoxalase-I activity, a kinetic study in 5 day old callus, after red light irradiation, showed that at 4 hrs there was a 68% increase in stimulation of glyoxalase activity compared to the dark controls and the increase in stimulation continued up to 24 hrs. However R/FR reversibility was not seen for glyoxalase enzyme and in fact there was a 35% increase in enzyme activity in callus irradiated with FR light alone, suggesting involvement of low energy response
like in cell proliferation studies. When R and FR light were given for 5 min per day for 8-days and enzyme activity checked on 9 day, again no R/FR reversibility was seen and FR irradiation alone showed a stimulation of 42% over the corresponding dark control and R+FR+R showed a stimulation of 100% in the glyoxalase activity.

9. The glyoxalase-I activity was also stimulated after irradiation of callus with blue light. At 4 hrs after irradiation the stimulation in the enzyme was 150%. Infact the enzyme activity increased even at 15 to 30 min after irradiation. Based on earlier work from this laboratory Das et al. (1987) proposed a model. According to the model the initial events of PI turnover and calcium release triggered by light could in turn affect the glyoxalase-I activity. To investigate further on this model, in the present work detailed experiments were done on the role of light on phosphoinositide turnover and Ca^{2+} haemoestasis and to check if a change in these in turn would affect glyoxalase-I activity and cell proliferation.

10. Light triggered PI turnover. This was checked by separating PI, PIP and PIP_2 through Dowex formate anion-exchange chromatography and also by TLC. In TLC the data were quantified using a densitometer scanner.
In white light grown callus there was generally low level of PI, PIP and increased level PIP$_2$. The percentage increase in PI was 62%, for PIP was 69% and in PIP$_2$ it was 102%. In red light there was a decrease in PI and PIP levels whereas in blue light there was an increase in PIP$_2$. In the time kinetic experiments of 15 min and 30 min, a change in PI turnover was seen both in red and blue light and there was an increase in PIP$_2$ level. The differential change in the levels of PI, PIP and PIP$_2$ in red and blue light treatments suggests different site of action for the turnover. There was very high concentration of IP$_3$ released in blue light treated callus.

11. Calcium uptake was affected by light. In irradiated cultures the calcium uptake was sustained at a higher level.

12. Lithium at 5 mM concentration inhibited PI turnover. This resulted in lowered glyoxalase-I activity and cell proliferation. The inhibition in cell proliferation and glyoxalase-I activity by lithium was only slightly reversed by A23187. However, when given alone A23187 mimicked the effect of light in stimulating glyoxalase-I and cell proliferation. These experiments showed that the PI turnover and Ca$^{2+}$ is involved in signal
transduction pathway in light mediated stimulation of glyoxalase-I and cell proliferation.

13. There was more increase in glyoxalase-I activity in callus grown an all hormone medium in presence of light than in dark grown callus and this was reflected in cell proliferation. However, in callus grown on GA3 medium alone there was a decrease in glyoxalase-I activity both under light and dark conditions. Whereas in dark grown cultures, cell proliferation could be correlated with decrease in glyoxalase-I activity in light grown cultures the correlation was not seen. Experiments of this nature do suggest that some parameters other than glyoxalase may also influence cell proliferation under certain conditions. It is possible that the level of MG could be regulated in these conditions by affecting its synthesis.

14. A cell lines was isolated individually from the proliferative callus of *Amaranthus* growing in the hormone medium. The rate of proliferation of this cell line differed from the parent line used in the present study. This cell line proliferated faster and therefore was called fast proliferating cell line (FPCL). On comparative basis, the other line was called slow proliferating cell line (SPCL). These cell
lines maintained their different rate of proliferation even though they were inoculated in the same medium and kept for same period of time (8 days) for proliferation. The cell lines could be maintained for many subsequent generations. These studies suggest that it should be possible to select cell lines with variable rates of cell division and therefore could provide efficient material for biochemical and molecular studies in future.

15. The proliferative callus of *Amaranthus* is a recalcitrant type and seldom regenerates into plantlets. There are very few reports on *in vitro* cultures of *Amaranthus* and hardly any on regeneration of callus. In attempts to regenerate, hypocotyl callus of *Amaranthus* by manipulating hormone composition in different media and changing other parameters, different types of responses could be achieved. Production of anthocyanin pigments and chlorophyll synthesis could be achieved by manipulating various combination of hormone. Anthocyanin production was seen in callus growing in MS1/2 + NAA (0.2 ppm) + BAP (0.5 ppm) + Picloram (8 uM) + 1/10th myoinositol and in MS1/2 + coconut milk (15%) + Zeatin (0.5 ppm) medium. In the same medium chlorophyll synthesis was also achieved. In some cultures, mixed callus producing anthocyanin
and chlorophyll was obtained and these calli could be isolated into anthocyanin producing and chlorophyll synthesising callii.

16. In morphological differentiation, direct regeneration of shoots were got from 10 day old hypocotyl on MS + Kn (0.5 ppm) + NAA (0.1 ppm) medium, and MS + NAA (0.2 ppm) + Kn (3 ppm) medium. Direct regeneration of roots were achieved from 10 day old hypocotyl on MS1/2 + NAA (1 ppm) + 1/10° myoinositol + 0.1% activated charcoal.

17. Using young inflorescence as explants, vegetative and reproductive structures from floral buds were produced. This effect was regulated by cytokinin concentration. At 3-6 mg/l, Kn favoured the formation of vegetative structures which could be developed in plantlets. The regenerated plantlets from inflorescence flowered in vitro.

18. Direct rooting was observed in the callus growing on the medium MS1/2 + NAA (0.2 ppm) + BAP(0.5 ppm) + 0.1% activated charcoal. Coconut milk (15%) when added to the above medium produced healthy multiple rooting in the callus. Addition of picloram at concentration of 8 μM increased multiple rooting.

19. Various treatments were given but shoot formation was obtained at a very low percentage in the callus growing
on MS1/2 basal + 1AA (0.3 ppm) + Kn (3 ppm) + 1/10th myoinositol medium. In some cultures the green calli became nodular and showed embryoid like structures in the medium MS1/2 + Coconut milk (15%) + Zeatin (0.5 ppm) after several transfers. The embryoid like structures, however, failed to regenerate into plants.