CONCLUDING REMARKS
From the investigations incorporated in the Chapter 1-3 the following conclusions have been drawn:

It was necessary to standardize an uniform cultural procedure so that the different experimental tissue could be grown in an identical cultural environment. The results from different replicas of each set of experiment incorporated in this thesis, though originate from a biological system, showed minimum differences. It was possible only because the experiments were performed following a critically standardized technique. The experiment with the selection of inoculum weight may be cited as an example. As the experiments were performed in a defined medium it was easier to make interpretation of the results. If the experiments could have been done in media containing coconut milk or casein hydrolysate their remained chances of having interference of some so far unknown compounds with the treated tissue leading to an uncritical assessment of the results.

Nigella tissue is basically IAA requiring and the IAA requirement of the tissue was judged by the fact that (1) IAA, only in its physiological concentration (0.1 mg/l) in the medium could activate the growth, and (2) IAA delayed the ageing of the tissue. Further, like most other tissue Nigella is dependent on vitamins, specially of B group, and the zeatin. It was also noted that vitamin and zeatin acted in a synergistic way and some future plan of work may be made for the study of vitamin-zeatin interactions using Nigella tissue.
Basic information obtained from these series of investigations is that callus growth is directly proportional to the IAA-oxidase and inversely proportional to the level of endogenous IAA. IAA itself enhanced the activity of IAA-destroying enzyme in the tissue only at its physiological concentration (0.1 mg/l). Such phenomenon has been considered to be due to an adoptive synthesis of IAA oxidizing enzymes. More was the synthesis of this enzyme more was the growth. Does it indicate that the reactive intermediate in IAA action is an IAA-oxidation product formed in presence of IAA-oxidase? Tuli & Moyed (1969) suggested that 3-methylene oxindole (3-MO), the oxidized product of IAA was the active form of IAA. Low conc. of 3-MO was considered to stimulate growth in their system. That IAA may require and an in vivo oxidative activation in order to express its biological function was also shown by Meudt (1967). Biological inactivation or activation of IAA effected by peroxidase may be governed more by ultimate high bounding nucleophile (Bednar et.al., 1976). Thus, the background informations and the results of the present experiment can be used as circumstantial evidences to conclude that perhaps the phenomenon of IAA oxidation is the prerequisite for the activation of growth. Due to the oxidation of IAA in presence of high IAA-oxidase activity (in most of the experiment) the level of endogenous IAA fell in a rapidly growing tissue. Increased IAA-oxidase was shown to be associated with meristematic and actively growing tissue (Briggs et.al., 1955; Ockerse et.al. 1970 & Meudt 1970). Schneider & Wightman (1974) showed that level of free IAA was in
turn regulated by enzymatic degradation catalyzed by indole-3-acetic acid oxidase. Also, it was stated that, increase of IAA-oxidase may cause destruction of IAA, therefore, affecting growth, (Omran, 1980). The phenomenon of occurrence of inverse relationship between IAA and IAA-oxidase reported in the present thesis received supports also from the works of Pilet (1967); Kerstetter & Keitt (1966); Galston (1956) etc.

The application of synthetic auxins jointly with IAA in the medium raised the level of endogenous IAA in the tissue. It was stated that specially NAA competes with IAA in binding sites. Ray (1977) reported that the dissociation co-efficient values of IAA-binding sites and NAA-binding sites for IAA were same, and NAA/IAA bind to the same site of membrane. But, IAA-oxidase activity in the system is related only to the amount of IAA to be utilized for growth. That is why, when a system uses synthetic auxins for growth, the IAA become surplus and accumulate in the tissue in a higher level. Similarly, IAA-ox. also is not triggered though a tissue grows normally; and for a similar G.V., rate of IAA-oxidase activated in the system in presence of IAA is more than that stimulated by synthetic auxins. The synthetic auxins are not subject to auxin indole path way, and it was reported that NAA or 2,4-D inhibits the reduction of 3-MO to the inner 3-methyl oxindole (Moyed & Williamson, 1967). Thus, it may also be true that IAA accumulation in the tissue was due to the inhibition of IAA-oxidation pathway by 2,4-D or NAA. Thus, not necessarily always IAA accumulation is related to a poor growth of tissue.
From the experiment with different spectrum of light, it was noted that the tissue behaved differentially at the influence of different spectrum of light. But in each case the growth stimulation was associated with activation of IAA-ox. with consequent decrease of level of endogenous IAA in the tissue. Maximum growth activity in near blue region of the spectrum was possibly due to the stimulation of riboflavin at that particular range of wavelength. Further, the detection of riboflavin in the aqueous extracts of tissue strengthened the idea that riboflavin might be the photoreceptor. And this concept has received support from the results of the experiments with riboflavin itself. Also, it has become suggestive that IAA oxidase is not the only agent catalyzing the utilization of IAA by the system for growth, because the interaction of enzyme activity in tissue grown in medium, containing riboflavin and IAA in different conditions, was insignificant (Table-A45). An alternative way of IAA activation is the photooxidation of IAA mediated by the photo-receptive pigments provided there is an appropriate environmental condition. Again, it was clarified that it is the riboflavin rather than the carotenoids which mediate the photosensitisation of IAA.

However, the cooperation of riboflavin and carotenoids thus providing a composite action spectrum, remains to be resolved in future. However, facts about the involvement of riboflavin was reported by Galston as early as in 1949. Thus most important phenomenon related to growth is the oxidation of IAA may it be by enzymes or by light mediated processes. Mn** mediated oxidation
of IAA on the other hand, indirectly evidenced (1) by the presence of low level of endogenous IAA and (2) by observed enhanced activity of IAA-oxidase also support the earlier proposition. Although, this is a clear case of enzyme mediated activation of IAA. The fact that Mn$^{++}$ increased the rate of enzymatic IAA oxidation was shown earlier by Kenten (1955) and others.

Callus tissue of Nigella could be grown in an IAA free medium provided the medium contained zeatin. But a reciprocal condition was not favourable for the same tissue. It indicates that zeatin may induce the tissue to synthesize its own IAA but IAA cannot induce the tissue to get its own cytokinin. In an IAA free medium, the calli might have an equally good growth value but for the induction of organogenesis in a tissue, presence of both zeatin and IAA was necessary. Again, IAA and zeatin should be allowed to interact in their specific concentrations. It was observed that such interaction showed a poor manifestation in dark. But in 12 hrs. light condition there was no organogenesis or a tendency of very poor organogenesis was noticed. Rate of organogenesis increased only when appropriate photoperiods were employed. Root formation was favoured by a long night treatment while the shoot bud formation was favoured by a long day condition.

Thus, the influence of photoperiod in addition to those of chemical milieu of the cultural environment was the determining factors for the induction of organogenesis. Blue light mediated organogenesis was shown earlier by Seibert et al. (1975).
In conclusion, we can propose that for the induction of morphogenesis the phytochromes are involved and there are already many reports indicating such involvements.

A future plan of experiment can be designed to investigate the mode of control that phytochromes operate and induce morphogenesis through hormonal interactions. It may be that in the regulation of organogenetic pattern there is a critical relationship between IAA and IAA-oxidase activity in the tissue. That is why the calli undergoing rooting incorporate a high endogenous IAA and also a high IAA-oxidase activity in comparison to a shoot forming tissue, again, the case is different in calli not differentiating at all. Presence of such specific relationship indicates that IAA oxidase helps to maintain the level of residual IAA in the tissue or it determines the amount of IAA to be utilized for a specific purpose. We know several reports of phytochrome mediated synthesis of phenols, which in turn may control the IAA-oxidase activity, and IAA-oxidase in turn may determine the level of endogenous IAA in tissue.

IAA-oxidation has been singularly interpreted, excepting a few occasions, as resulting in the biological inactivation of IAA presumably explaining the growth inhibition. But we would like to suggest that possibly oxidative transformation of IAA in the tissue might contribute to the biological activity of IAA. IAA-oxidase activity in a system actually activates IAA rather than acting as a simple IAA-destroying factor. Similar is the case with photoactivation of IAA.